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CONTENTS

The anaerobic dissimulation of glucose by Fusarium lini
VINCENT W. COCHRANE 1

Studies on the mechanics of growth in the common mushroom, Agaricus campestris
JOHN TYLER BONNER, K. KENT KANE AND RAPHAEL H. LEVEY 13

Simultaneous self-stimulation and self-inhibition of uredospore germination.....C. E. YARWOOD 20

Physiological studies of some fungi associated with pole blight of western white pine.....CHARLES D. LEAPHART 25

The association of yeasts with certain bark beetles
M. SHIFRINE AND H. J. PHAFF 41

The perfect stage of Hormodendrum pedrosoi
CESAR A. FUENTES AND FREDERICK A. WOLF 56

Studies on Trichophyton tonsurans. I. The taxonomy of T. tonsurans.....LUCILLE K. GEORG 65

Undescribed species of Synchytrium.....JOHN S. KARLING 83

Type studies of the polypores described by Karsten
JOSIAH L. LOWE 99

Uredinales parasitizing grasses of the tribe Chlorideae
JOE F. HENNEN AND GEORGE B. CUMMINS 126

The occurrence of Arachniotus citrinus in soil
BILLY WEST AND LIBERO AJELLO 163

Notes and Brief Articles 166

Bonorden: Histoplasma. Snell and Dick: Polypores. Ragab: Fungi of Egypt. A new microbiological journal.

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XLVIII JANUARY–FEBRUARY 1956 No. 1

THE ANAEROBIC DISSIMILATION OF GLUCOSE BY FUSARIUM LINI [1]

VINCENT W. COCHRANE

(WITH 2 FIGURES)

It has long been recognized that the metabolism of glucose by *Fusarium* species under conditions of restricted aeration is similar in at least some respects to that of yeast (**34**). In growing cultures the ratio of ethanol to carbon dioxide often approaches unity (**1, 2, 27**), although the ability of the fungus to utilize ethanol (**10, 33**) means that exact ratios cannot reproducibly be obtained in long-term growth experiments. Other evidence from which it might be inferred that the yeast pattern is followed includes the effect of thiamine (**6, 35, 36**), the effect of added diphosphopyridine nucleotide (**12**), and the demonstration of carboxylase and cocarboxylase in dried cells of *Fusarium tricothecioides* (**32**).

It must be recognized that this evidence is inconclusive, and it has been vigorously maintained that the classical Embden-Meyerhof pathway of yeast metabolism is of little or no importance in *Fusarium* (**21, 22, 25, 26**). The evidence, largely negative, will be summarized later in this paper.

Experiments with resting cells and with extracts have been designed to test the hypothesis that the anaerobic formation of ethanol and carbon

[1] This investigation was supported by a research grant (G-3154) from the Division of Research Grants and Fellowships of the National Institutes of Health, and by a grant-in-aid (MET-18) from the American Cancer Society on recommendation of the Commitee on Growth of the National Research Council.

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dioxide by *Fusarium lini* Bolley [2] can be explained on the basis of the Embden-Meyerhof scheme alone. The choice of criteria is dictated in part by convenience, in part by the nature of the organism; thus the presence of active phosphatases in the extracts rules out some types of experiment. The following characteristics of the Embden-Meyerhof sequence have been selected for study with the purpose of determining whether *F. lini* meets the requirements of the basic hypothesis:

1. Resting intact cells form two moles of ethanol and two of carbon dioxide per mole of sugar utilized.

2. Carbon atoms 3 and 4 of glucose appear as carbon dioxide; carbon atoms 1 and 6 do not appear significantly in carbon dioxide (**15**).

3. Fructose-1,6-diphosphate is split by aldolase to yield triose phosphates, determinable as alkali-labile organic phosphorus.

4. The oxidation of glyceraldehyde-3-phosphate, derived from fructose-1,6-diphosphate, involves the reduction of diphosphopyridine nucleotide and is poisoned by iodoacetate.

5. 3-Phosphoglyceric acid, in the presence of adenosine triphosphate, can reoxidize reduced diphosphopyridine nucleotide, the reactions being catalyzed by phosphoglyceryl kinase (**4**) and triose phosphate dehydrogenase.

6. Phosphoglyceric acid is converted to pyruvic acid, the conversion being inhibited by fluoride.

7. Pyruvic acid is decarboxylated to acetaldehyde and carbon dioxide by pyruvic decarboxylase.

8. Ethanol dehydrogenase converts acetaldehyde to ethanol with the simultaneous reoxidation of reduced diphosphopyridine nucleotide.

MATERIALS AND METHODS

Fusarium lini Bolley, strain No. *9593* of the American Type Culture Collection, was grown routinely on agar slants containing, per liter: glucose 10 gm and Difco nutrient agar 23 gm. A water suspension prepared from these slants after 14–21 days of growth was used to inoculate 100 ml portions of the growth medium in 500 ml Erlenmeyer flasks. Inoculated flasks were incubated 3 days on a reciprocating shaker at 25° C.

Two growth media, a synthetic and a complex, were used. The synthetic medium contained, per liter: glucose 10 gm, $(NH_4)_2HPO_4$ 2.6 gm, KH_2PO_4 1.5 gm, and $MgSO_4 \cdot 7H_2O$ 0.75 gm. The complex medium contained, per liter: Difco proteose peptone No. 3, 20 gm, Difco

[2] *Fusarium oxysporum* f. *lini* (Bolley) Snyder & Hansen.

beef extract 3 gm, Difco malt extract 3 gm, Difco yeast extract 3 gm, and glucose 10 gm. Glucose for both media was sterilized separately and added aseptically.

After the 3 days the cells were filtered off on Whatman No. 41-H paper on a Buchner funnel and washed twice with water. For experiments with intact cells the wet cells were weighed and suspended in 4 weights of water.

Cell-free preparations were made by grinding the damp cells in a chilled mortar with 3 weights of powdered Pyrex glass (40 mesh). After grinding, the broken cells were suspended in a solution containing 60 mg cysteine hydrochloride dissolved in 10 ml of 0.1M $KHCO_3$; cell debris was removed by centrifugation (10 min. at 3000 × G). It was found that the cysteine is essential, and that it is in fact distinctly advantageous to wash the cells before grinding in the same cysteine solution used for the final suspension. The function of the cysteine is, presumably, to protect enzymes against inactivation by heavy metals.

Ethanol was determined enzymatically (**3**), phosphate by the method of Gomori (**11**), and coenzyme reduction by changes in absorption at 340 mμ in a Beckman Model DU spectrophotometer. In experiments with labeled glucose the cells were killed with acid at the end of the reaction and the carbon dioxide previously evolved was collected in concentrated NaOH introduced into the sidearm. The Na_2CO_3 was then converted to $BaCO_3$, which was counted in a methane flow proportional counter.

RESULTS

Metabolism of Glucose by Intact Resting Cells

Using resting cells in a short-term experiment, it was possible to confirm earlier claims that glucose is converted, as in yeast, to two moles of ethanol and two of carbon dioxide (TABLE I). Cells for this

TABLE I

THE FERMENTATION OF GLUCOSE BY F. LINI

Substrate*	CO_2, μM	Ethanol, μM	Ethanol / CO_2
Glucose, 5 μM	10.0		
Glucose, 10 μM	15.7		
Glucose, 20 μM	38.4	42.6	1.11
Glucose, 40 μM	76.5		

* Each Warburg flask contained: phosphate buffer (pH 6.4) 0.017 M; $MgCl_2$ 10 μM; washed cells 300 mg; and water to 3.2 ml. Temperature 30° C, gas phase N_2, time 240 min. Endogenous values (3.2 μM CO_2, 1.5 μM ethanol) subtracted.

TABLE II

THE DISTRIBUTION OF GLUCOSE CARBON DURING FERMENTATION *

Substrate	CO_2, μM**	Specific activity, mμc/mg carbon		Total activity recovered as CO_2, percent
		Glucose	CO_2	
Glucose-1-C^{14}	15.7	13.1	0.9	1.80
Glucose-3,4-C^{14}	19.6	10.3	16.9	53.1

* Cells grown on synthetic medium. Each Warburg flask contained: phosphate buffer (pH 6.8) 30 μM, $MgCl_2$ 10 μM; washed cells 400 mg.; labeled glucose 10 μM; and water to 3.2 ml. Evolved CO_2 was collected in 20 percent NaOH at the conclusion of the experiment. Temperature 30° C, time 210 min., gas phase nitrogen.
** Endogenous (72 μL) subtracted.

experiment were grown on the synthetic medium, but other experiments with cells from the complex medium yielded the same result.

Results with specifically C^{14}-labeled glucose are presented in TABLE II, and conform to expectations, in that carbon atom 1 of glucose contributes negligibly to the carbon dioxide, and carbons 3 and 4 appear as carbon dioxide. However, the recovery of activity is anomalously low. The glucose-3,4-C^{14} used has a specific activity of 10.3 mμc. per mg carbon, or 30.9 mμc. per mg of carbons 3 and 4. The specific activity of the carbon dioxide liberated, 16.8, is substantially less than this expected value of 30.9. A similar phenomenon has been observed in the recovery of C^{14}-labeled acetate from the fermentation of xylose-1-C^{14} by *F. lini* (8), and is at present not understood.

Enzymes of Cell-Free Preparations

In all of the enzyme experiments the complex medium was used for the reasons that cell yields are greater and the cells are more easily broken by grinding than is the case when the synthetic medium is used.

Aldolase, which splits fructose-1,6-diphosphate to a mixture of the triose phosphates, was determined (TABLE III) by the formation of

TABLE III

ALDOLASE IN FUSARIUM LINI

Substrate*	Time, min.	Alkali-labile phosphorus, μ atoms
None	30	0.3
Fructose-1,6-diphosphate, 54 μM	0	1.5
Fructose-1,6-diphosphate, 54 μM	30	20.2

* Complete system: cell-free extract 1.0 ml; *tris*(hydroxymethyl)aminomethane buffer (pH 8.5) 150 μM; hydrazine sulfate 160 μM; water to 4.0 ml. Reaction stopped at time shown by addition of 0.4 ml. 50 percent trichloracetic acid. Temperature 25° C.

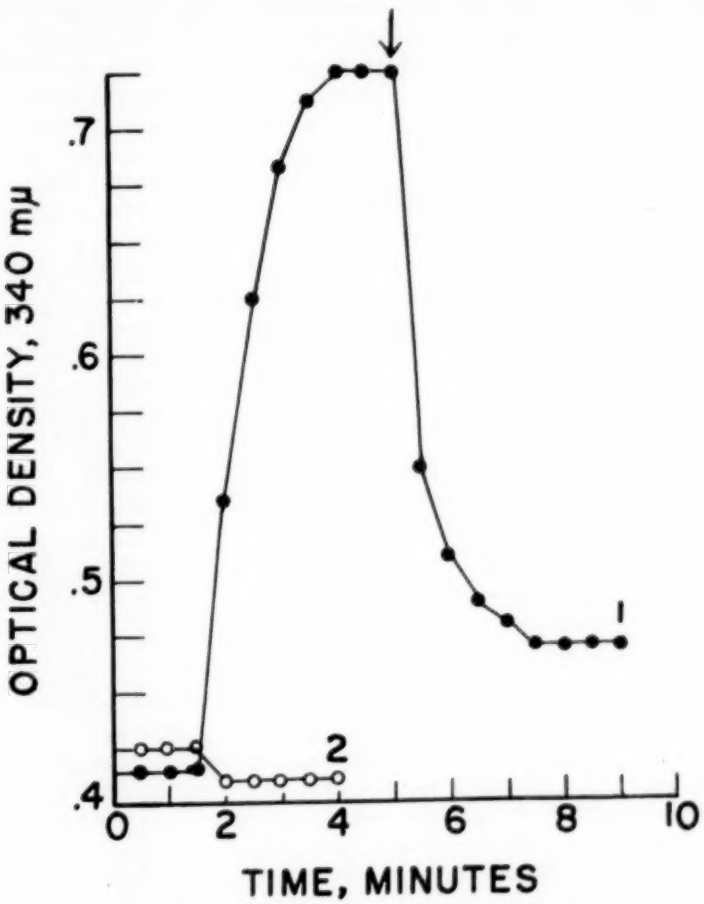

FIG. 1. Aldolase, triose phosphate dehydrogenase, and ethanol dehydrogenase in extracts of *F. lini*. Cuvettes contained: cell-free extract 0.1 ml; veronal buffer (0.03 M, pH 7.9) 1.5 ml; sodium arsenate 54 μM; sodium fluoride 60 μM; diphosphopyridine nucleotide 0.5 μM; fructose-1,6-diphosphate 12 μM, added at 1.5 min.; and water to 3.0 ml. Acetaldehyde (30 μM) added at time shown by arrow.

Curve 1: no inhibitor.
Curve 2: iodoacetate 0.001 M.

alkali-labile organic phosphorus compounds during incubation in the presence of hydrazine as a trapping agent (**29**). From the data of TABLE III, it is evident that about 19 percent of the substrate was converted to product; this agrees closely with the aldolase activity in extracts of *Streptomyces coelicolor* as measured by the same method (**5**).

Three enzymatic reactions are demonstrated by the spectrophotometric data of FIG. 2. Fructose-1,6-diphosphate is split by aldolase to triose phosphates; the oxidation of one of these, 3-glyceraldehyde phosphate, is evidenced by the concomitant reduction of diphosphopyridine nucleotide and a change in absorption at 340 mμ. When acetaldehyde is added in considerable excess the reduced coenzyme is reoxidized by the conversion of acetaldehyde to ethanol; this last reaction is catalyzed by ethanol dehydrogenase. The main reaction, oxidation of triose phosphate, is inhibited completely by iodoacetate. Data not shown indicate that, as expected, the omission of fluoride has no effect. No reaction occurs if the fructose-1,6-diphosphate is replaced by free glucose or by fructose-6-phosphate, with or without adenosine triphosphate.

The reactions leading from triose phosphate to phosphoglyceric acid may be summarized as follows:

(1) 3-phosphoglyceraldehyde + H_3PO_4 + $DPN^+ \rightarrow$ 1,3-diphosphoglyceric acid + DPNH + H^+

(2) 1,3-diphosphoglyceric acid + adenosine diphosphate → 3-phosphoglyceric acid + adenosine triphosphate.

Reaction (1) is catalyzed by triose phosphate dehydrogenase, reaction (2) by phosphoglyceryl kinase (**4**). A specific test for the activity of the latter enzyme consists in reacting an excess of phosphoglyceric acid and adenosine triphosphate in the presence of reduced diphosphopyridine nucleotide (DPNH) and the enzyme mixture. The effect of these additions is to reverse both reactions, and thereby to reoxidize the reduced diphosphopyridine nucleotide; the progress of the reaction can be followed spectrophotometrically at 340 mμ. Fluoride is added to prevent any forward metabolism of phosphoglyceric acid.

In *F. lini* this assay for phosphoglyceryl kinase (FIG. 2) is somewhat complicated by endogenous reoxidation of the reduced diphosphopyridine nucleotide, but the sharp drop on addition of adenosine triphosphate (ATP) may be taken as evidence for the reversal of reactions (1) and (2). As expected, iodoacetate inhibits. In control experiments, the sum of optical effects resulting from the addition of adenosine triphosphate and phosphoglyceric acid separately was invariably less than 50 percent of that resulting from the addition of both.

In the Embden-Meyerhof pathway phosphoglyceric acid is converted to pyruvic acid in three reactions. The second of these, conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid, is catalyzed by enolase and is strongly inhibited by fluoride in the presence of phosphate. The third step, conversion of phosphoenolpyruvic acid to pyruvic acid, requires a phosphate acceptor, *e.g.*, adenosine diphosphate.

Experimentally, the liberation of carbon dioxide was used as an index of the metabolism of phosphoglyceric acid; this is practicable in

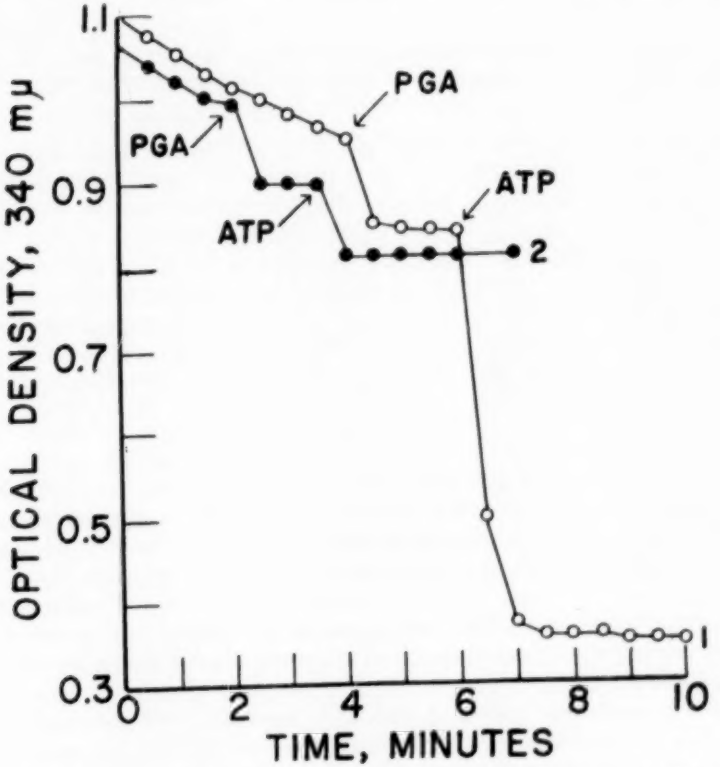

FIG. 2. Phosphoglyceryl kinase in *F. lini*. Each cuvette contained: cell-free extract 0.1 ml; veronal buffer (0.03 M, pH 7.4) 1.5 ml; sodium fluoride 60 μM; reduced diphosphopyridine nucleotide 0.4 μM; and water to 3.0 ml. Phosphoglyceric acid (PGA) 10 μM, and adenosine triphosphate (ATP) 10 μM, added as shown by arrows.

Curve 1: no inhibitor.

Curve 2: iodoacetate 0.001 M.

TABLE IV

THE METABOLISM OF PHOSPHOGLYCERIC ACID AND PYRUVIC ACID BY F. LINI

Substrate*	Fluoride, μM	Adenosine diphosphate, μM	Carbon dioxide, μM	
			Total	Endogenous subtracted
None	—	6.0	1.8	0.0
Phosphoglycerate, 22 μM	—	—	4.1	2.3
Phosphoglycerate, 22 μM	—	6.0	24.8	23.0
Phosphoglycerate, 22 μM	90	6.0	0.7	−1.1
Pyruvate, 36 μM	—	—	35.2	33.4

* Basal system: potassium phosphate buffer (pH 7.0) 30 μM; $MgCl_2$ 10 μM; cell-free extract 1.0 ml; water to 3.2 ml. Temperature 30° C, gas phase nitrogen, time 120 minutes.

F. lini by reason of the fact that pyruvic acid is rapidly decarboxylated (TABLE IV). The other data of TABLE IV show that phosphoglyceric acid yields stoichiometric amounts of carbon dioxide, that the reaction is inhibited by fluoride, and that adenosine diphosphate is required as a phosphate acceptor. Adenosine triphosphate replaces adenosine diphosphate, presumably being converted to it by phosphatases present in the extract. Glucose, however, does not serve as a phosphate acceptor in place of adenosine diphosphate. The rapid decarboxylation of pyruvate confirms the finding of Tytell and Gould (**32**) on *F. tricothecioides*.

DISCUSSION

From these data it is concluded that the fermentation of glucose by *F. lini* can satisfactorily be accounted for on the basis that the Embden-Meyerhof pathway of anaerobic hexose catabolism is followed, and there is nothing in the data to indicate that some other pathway must be postulated. The most convincing evidence is (1) the distribution of isotopic carbon from differentially labeled glucose, (2) the occurrence of particular enzymes, and (3) the metabolic reactions of phosphoglyceric acid.

As mentioned earlier, it has been suggested that the Embden-Meyerhof sequence is of negligible importance, if indeed it occurs at all, in the formation of ethanol from glucose by *Fusarium* species. The most recent summary of this point of view is that of Nord and Weiss (**26**), who cite the following evidence:

1. It is not possible to detect uptake of inorganic phosphorus from the medium by growing cells of *F. lini* until 2–5 days after the onset of fermentation.

2. The content of adenosine triphosphate in *F. oxysporum* is less than that usually found in yeast and bacteria.

3. Addition of adenosine triphosphate to the medium has no discernible effect on metabolism.

4. Phosphoglyceric acid cannot be isolated from living cells metabolizing glucose in the presence of fluoride.

5. Glycerol is not produced during pentose fermentation with sulfite.

6. Phosphoglyceromutase cannot be detected.

7. *F. solani* tolerates concentrations of fluoride which normally inhibit the yeast fermentation.

8. 3-Phosphoglyceric acid is not utilizable as a carbon source.

Most of these observations are negative, and some, especially the first, fourth, and sixth, may only reflect technical difficulties. In the case of the first, reference to the original work (**23**) shows that an attempt was made to measure the disappearance of inorganic phosphate from a medium so high in phosphate (110–132 μM per ml) that small differences might escape detection. The second point—the low content of adenosine triphosphate—can hardly be taken as an argument against the occurrence of fermentation; the important finding here is that some adenosine triphosphate is present. Failure of adenosine triphosphate to affect the metabolism of intact cells is not surprising, in view of the known occurrence of phosphatases in fungi and in view also of the strong probability that the intact cell is impermeable to the compound. Permeability difficulties can easily account also for the lack of effect of fluoride on intact cells (Point 7) and for the failure of phosphoglyceric acid to serve as a carbon source (Point 8). Failure of glycerol to appear during pentose fermentation (Point 5) is difficult to interpret in view of the incompleteness of our knowledge of pentose metabolism and enzymatic equilibria, but the finding has no necessary bearing on the normal course of hexose catabolism.

The present work has relied on rather different methods, and little direct refutation of these points is offered. However, the apparent noninvolvement of phosphoglyceric acid, a key element of the criticisms of Nord and Weiss (Points 4, 6, 7, and 8), is directly contradicted by the studies reported here on cell-free extracts. In view of permeability limitations, it is hardly likely that methods involving intact cells can be relied upon in the study of the metabolism of phosphorylated intermediates. These studies on extracts are supplemented by the results with intact resting cells (TABLES I and II), which conform completely to the Embden-Meyerhof pathway.

It can never be said, of course, that an organism does not have an

additional pathway which is used under conditions different from those chosen for experiment. With regard to the fermentation of glucose by *F. lini*, it can, however, be held with some certainty that there is at present no evidence which requires the postulate of a new, non-phosphorylative, pathway. The same conclusion has been reached for animal cells (**7**) and for yeast (**18**).

Evidence for the occurrence and importance of the Embden-Meyerhof pathway in fungi and actinomycetes is slowly accumulating. Enzymatic studies include rather complete analyses of *Aspergillus niger* (**14**) and *Stereptomyces coelicolor* (**5**); in the latter organism one essential step of the sequence is lacking and glucose is therefore not metabolized anaerobically. Important enzymes of the pathway have been found also in *Neurospora crassa* (**19, 20, 30, 31**) and *Penicillium notatum* (**17**). Isotope studies on *Rhizopus nigricans* (**9**) are consistent with the operation of a modified Embden-Meyerhof pathway in this organism. The distribution of tracer from labeled glucose during aerobic respiration in *Penicillium chrysogenum* (**16**) and during citric acid synthesis in *Aspergillus niger* (**28**) is similarly consistent with a considerable contribution of the Embden-Meyerhof pathway.

In its oxidative metabolism, of course, *F. lini* may or may not utilize the Embden-Meyerhof pathway. In this laboratory some evidence has been found that the "hexosemonophosphate shunt" (**13**) is enzymatically possible; the report (**24**) that *F. lini* forms gluconic acid raises still another possibility for aerobic metabolism.

SUMMARY

Resting cells of *Fusarium lini* Bolley ferment glucose anaerobically to equimolar amounts of ethanol and carbon dioxide, two moles of each product being formed per mole of glucose supplied. The distribution of glucose carbon is in accordance with the Embden-Meyerhof pathway of yeast and other organisms. Enzymes demonstrable in cell-free extracts include: aldolase, triose phosphate dehydrogenase, phosphoglyceryl kinase, carboxylase, and ethanol dehydrogenase. Other data are consistent with the view that the conversion of phosphoglyceric acid to pyruvic acid follows the Embden-Meyerhof pattern. The facts presently known do not require that a second, non-phosphorylative, pathway of anaerobic glucose dissimilation be postulated.

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WESLEYAN UNIVERSITY,
MIDDLETOWN, CONNECTICUT

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STUDIES ON THE MECHANICS OF GROWTH IN THE COMMON MUSHROOM, AGARICUS CAMPESTRIS [1]

JOHN TYLER BONNER, K. KENT KANE AND RAPHAEL H. LEVEY

(WITH 4 FIGURES)

Despite the fact that mushrooms have been an object of great interest to the naturalist for many centuries, and despite the fact that mushrooms have been cultivated since early times, it is surprising how little work has been done on their morphogenesis. De Bary (**1887**) has given a clear description of the sequence of events in a few species and since then there have been several noteworthy studies of the development of different mushrooms, each of which shows its special species characteristics. In the case of *Agaricus campestris*, which concerns us at the moment, Atkinson (**1906**) examined the early development of the gills and Hein (**1930a**, **1930b**) has revealed many details of the growth process, but it is difficult from these and other studies to obtain a comprehensive view of the development as a whole. More progress has been made recently by Borriss (**1934**) on the morphogenesis of *Coprinus lagopus* but much fundamental information on mushroom growth in general is still lacking and this study is a contribution toward obtaining a more complete picture of the development of *Agaricus campestris*.

MATERIALS AND METHODS

The type of mushroom used in all the experiments was the snow white strain of *Agaricus campestris* obtained through the kindness of Mr. Karl Knaust, from Knaust Brothers, Inc., Catskill, New York. The mushrooms were grown in small commercial flats at 18–20° C and at a humidity sufficient to prevent the soil from drying out.

For the histological work, fixation of whole or parts of mushrooms was done with 1 percent chrom-acetic for three hours. Dehydration was with tertiary-butyl-alcohol preceded by a series of ethyl alcohols. The material was embedded in paraffin, sectioned at 10 μ, and then stained with phloxine and fast green.

[1] This research was supported in part by funds of the Eugene Higgins Trust allocated to Princeton University.

Dry weights were made by placing the mushrooms in an oven at 105° C for fourteen hours.

RESULTS

The growth zone. It is well known (see, for instance, Magnus **1906**, Hein **1930b**, and Borriss **1934**) that the zone of rapid elongation during mushroom development lies in that part of the stalk directly below the cap. An attempt was made here to identify this region more precisely and follow it throughout the course of development. This was done by placing equidistant spots of carmine up the length of a mushroom at an early stage and then these were followed carefully throughout development.

The results may be seen in FIGS. 1 and 2. In FIG. 2, the shaded area is that in which the process of expansion is actually taking place and, therefore, the exact location and extent of the growth zone can be seen at a glance for the different stages.

It was found that small pieces of the mushroom, either total longitudinal slices or small transverse segments, could be cut out of a mushroom at stage B and that this piece, if marked with carmine and placed on moist agar, would undergo a small but definite expansion following the pattern indicated in FIGS. 1 and 2. This is consistent with Borriss' (**1934**) discovery that at an advanced stage in *Coprinus logopus*, isolated stems or caps elongate independently. It was even possible to excise the growth zone itself and it showed a fair expansion over a three-day period.

The cap was studied separately with the carmine dots and was found, after stage B, to expand in a gradient; the greatest expansion being at the margin and little or no expansion in the central portion.

Distribution, orientation and size change in the hyphae during development. The information obtained from a study of the paraffin sections of various stages of the mushroom growth have brought no new facts to light but they do confirm the observations of the early workers and they are pertinent here for a clear understanding of the mechanics of expansion described in the previous section.

From the very beginning of the button formation to one approximately 4 mm high (Stage A in FIG. 1) the hyphae are arranged throughout the mass in a most irregular tangle. There is some slight orientation in the gill region for the gills and the gap below the gills appears when the button is but 2 mm high.

When a button reaches a height of approximately 5 mm, the arrangement of the hyphae in the stalk region below the cap (i.e., the future

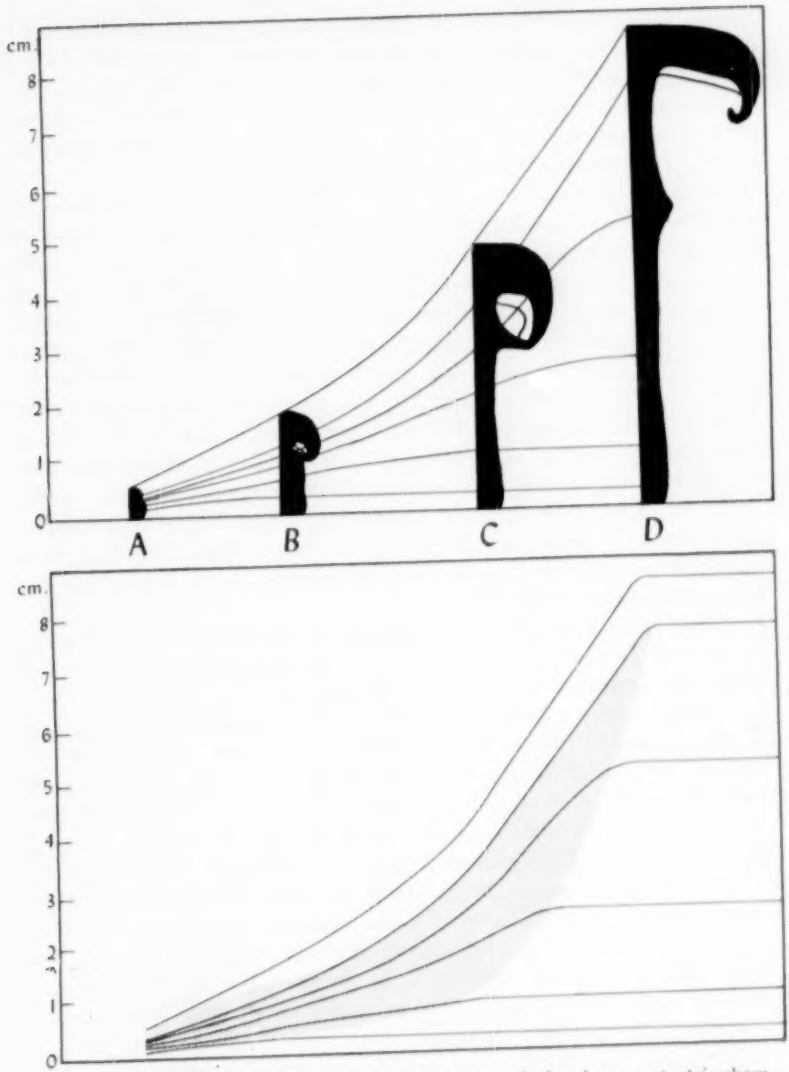


FIG. 1 (*above*). A diagrammatic growth curve of *Agaricus campestris* showing four different stages. The lines are drawn through homologous points. The horizontal axis is only a very rough approximation of the passage of time.

FIG. 2 (*below*). This is the same graph as above but those regions of the stalk that are in the process of elongation (i.e. the growth zone) lie within the shaded area. In this way it is possible at each stage of development to see the extent of the growth zone.

growth zone) begins to form in an oriented fashion, with the filaments lined up and down the stalk. By the time the button has reached 10 mm this orientation in the upper stalk region is complete. The base of the stalk remains a tangled mass of threads, and the cap appears likewise, although occasionally in a fortuitous longitudinal section, it is possible to see a considerable amount of radial orientation.

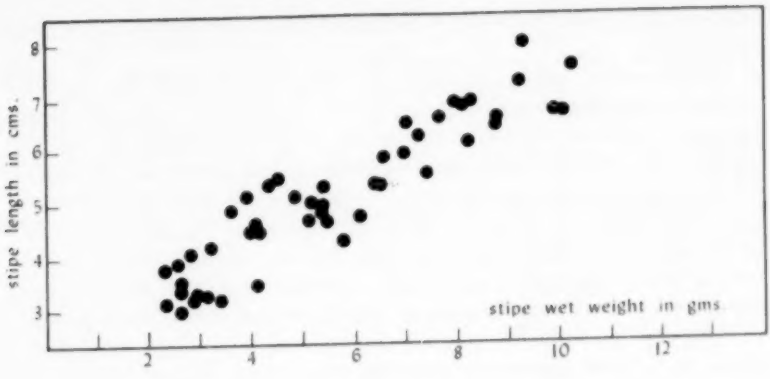
By stage B (FIG. 1) we have good evidence, which corroborates the early statement of De Bary (**1887**), that there is no further increase in the number of hyphae or the number of cells in the stalk region. Cell size measurements were made on an 18 mm and a 55 mm mushroom and the small increase in girth is paralleled by a corresponding increase of girth of the cells; also the expansion of the central cavity with further increase the girth in the middle region of the stalk. The increase in length is more difficult to analyze in that the cells in the growth region are consistently shorter than the ones below. For instance, in the 18 mm mushroom the average of 24 measured was 23 μ in the growth zone and 41 μ close to the base of the stalk (but above the tangled portion), while in the 55 mm mushroom, the corresponding values were 43 μ and 81 μ. If the extent of the growth zone is taken into account then there is a three-fold increase for both the total overall increase in height and the increase in length of the cells.

Presumably, then, all the arrangement of the hyphae has taken place by stage B (FIG. 1) and from there to the final mature size, there is simply an expansion of the cells in the filaments.

Further evidence for growth being by the elongation of the hyphae. If the heights of stalks of mushrooms at different stages of development are plotted against their wet weights, as was done with 49 mushrooms, the plot is reasonably linear (FIG. 3). This shows that of the three possible dimensions, one alone, that is, length, is being increased and therefore growth is largely an extension of the filaments.

In the case of the cap, the growth is radial, in two dimensions, therefore, if in the same mushroom the cap diameter is measured and then squared, and in turn plotted against the wet weight of the cap, one would again expect the relation to be linear. However, when this is done the scatter of the points is so great that it is impossible to demonstrate linearity with any certainty.

Evidence that the increase in size and weight is more than mere water absorption. We have now shown by two independent lines of evidence that growth in the stalk is, after stage B (FIG. 1), mainly a matter of cell elongation. Now comes the question of what happens to the cells so that they can lengthen; namely, is it a matter of



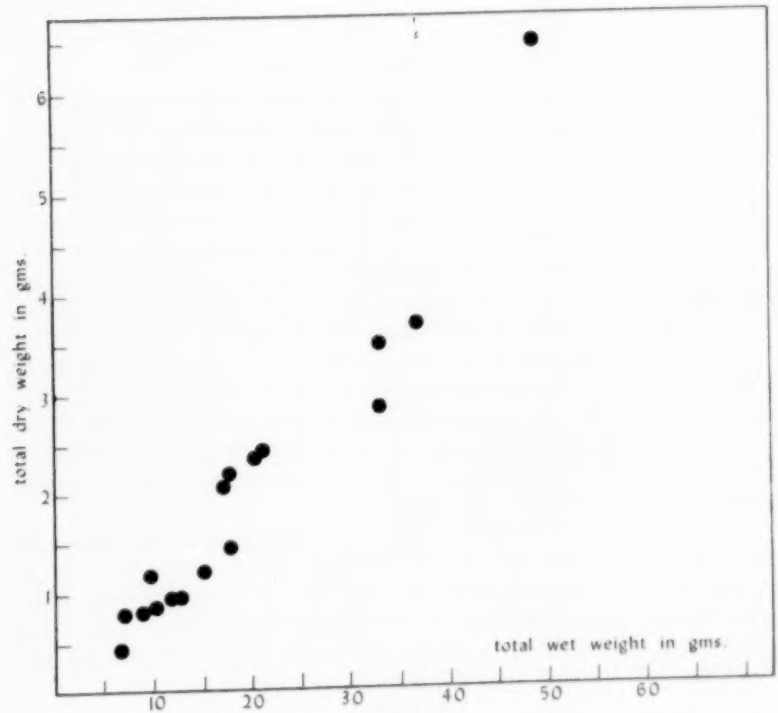


FIG. 3 (*above*). The length of the stipe is plotted against the wet weight of the stipe for 49 mushrooms.

FIG. 4 (*below*). The dry weight of 17 mushrooms is plotted against the wet weight of the same mushrooms.

simple water intake, or is there a passage of materials from the mycelium in the soil?

This question was tested simply by comparing the wet with the dry weight of the mushrooms. As can be seen from FIG. 4, the dry weight increases in direct proportion to the wet weight, or to put the matter another way, the dry weight/wet weight ratio remains the same throughout the extensive final growth period. This relation holds not only with the whole mushroom (FIG. 4) but when the cap and the stalk are measured separately. Therefore, it is clear that the normal growth in the soil is totally dependent on materials coming from the subterranean mycelium and that water is taken up only in proportion to these materials.

This fact undoubtedly accounts for the relatively slight expansions found in isolated pieces of mushroom where only a small increase in water intake can occur.

SUMMARY AND CONCLUSIONS

The growth of the mushroom *Agaricus campestrus* was studied with the aim of obtaining a clear description of the mechanics of the process. First, by means of measuring mushrooms marked with carmine dots at regular intervals, the details of the location of the growth region during different phases of growth were described (FIGS. 1, 2). From paraffin sections it was possible to show that, following an early period of randomly oriented hyphae, the hyphae became oriented longitudinally in the growth zone and that from approximately the 18 mm stage onward there is probably little or no increase in cell number (at least in the stalk), for the increase in cell size is sufficient to account for the total increase in size. The fact that increase in size is primarily by cell elongation in the stalk was supported by the weight-size relationships (FIG. 3). This elongation of the cells was then shown to involve a transport of materials from the mycelium in the soil since the dry weight increased proportionately with the wet weight (FIG. 4).

In conclusion, it should be pointed out that in a sense all the major problems of mapping out the overall shape of the mushroom and the laying down of the majority of the cells is done at a very early stage and that the subsequent three- to four-fold increase in size is merely the expansion of specific parts of this preformed bud, which involves the sucking up of nutrient materials from the soil mycelium.

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SIMULTANEOUS SELF-STIMULATION AND SELF-INHIBITION OF UREDOSPORE GERMINATION [1]

C. E. YARWOOD

(WITH 1 FIGURE)

Self-stimulation of spore germination (Ezekiel, **1930**; Smart, **1937**) and self-inhibition of spore germination (McCallan, **1930**; Yarwood, **1954**; Allen, **1955**) are uncommonly reported but probably not rare phenomena. Simultaneous self-stimulation and self-inhibition of the same spores has apparently not been reported. Of course simultaneous self-stimulation and self-inhibition can only be demonstrated if a separate criterion is used for each. In this study percent germination and length of germ tubes are the separate criteria used. Usually factors which favor relatively high percent germination also favor further growth of the germ tubes (Peltier, **1922**; Anderson, **1934**). The present report of simultaneous self-stimulation and self-inhibition is an exception to this rule but is really a combination of two phenomena of uredospores which have been previously reported independently (Ezekiel, **1930**; Yarwood, **1954**).

METHODS

Uredospores of *Uromyces phaseoli* were shaken from bean plants growing in the greenhouse to Petri dishes containing 10 ml of water agar with or without added chemicals. The range of spore dosage was from 0.2 to 160 spores per mm^3 of agar. Spore dosage is given as spores per unit volume rather than per unit area because it was found that spores per unit volume of agar was the dosage character which determined the resulting germination and germ tube length. This was tested by varying the number of spores per unit area with the agar thickness constant at about 1.6 mm, or less frequently by varying the thickness of the agar between 0.1 and 8 mm with the number of spores per unit area constant.

[1] While this manuscript was in press the self-inhibitor of *Puccinia graminis* has been identified as trimethylethylene in the following study: Forsyth, F. R. 1955. The nature of the inhibiting substance emitted by germinating urediospores of *Puccinia graminis* var. *tritici*. Canadian Jour. Bot. **33**: 363–373.

After the seeded plates had incubated for about 24 hours at 25° C in the dark, percentage germination and germ tube length were determined. For this record only spores not in contact with other spores were used. This was because many spores were in groups of 2 or more, and on thinly seeded plates these grouped spores produced longer germ tubes than did isolated spores, that is, they behaved in this respect as heavily seeded spores. For determination of spore dosage, however, both isolated spores and those in groups were counted. This is because the spores in groups were presumed to be as active in liberating the substances which reduced percentage germination and increased the length of germ tubes as were single spores.

RESULTS

Data for most trials showing the relation of spore dosage to percentage germination and germ tube length are given in FIG. 1.

For percent germination 50 spores per plate were counted and spores were considered as germinated if germ tubes were as long as the diameter of the spore. The length of germ tubes was measured with an eyepiece micrometer in which each division equalled 17 μ when used with the low power objective. Germ tubes were measured or estimated to the nearest micrometer division. Considerable estimation was necessary for much convoluted germ tubes. Ten random germ tubes were measured per plate. Because average length of germ tubes varied so slightly with considerable changes in spore concentration, most results are for spores at the high and low ends of the spore concentration series. The reason for the great variation in length of germ tubes for the same spore concentration is principally because most trials included chemical or other treatments which greatly modified (usually reduced) the length of the germ tubes. Usually chemical treatments which decreased the absolute length of the germ tubes, increased the relative difference in germ tube length due to spore concentration. For example, in one trial spores seeded at 0.4 and 124 spores per mm^2 on plain agar produced germ tubes averaging 600 and 790 μ, respectively, while spores seeded on plain agar to which had been added 0.1 cc of 0.1% concentrated H_2SO_4 produced germ tubes averaging 230 and 740 μ, respectively. The percent increase in germ tube lengths due to increased spore concentration was, therefore, 32 for the plain agar and 220 for the spores on acidified agar in this test.

At 13° C the difference in percent germination and germ tube length was much less than at 25°, though the absolute percent germination and absolute length of the germ tubes were as great or greater at 13° than at 25°.

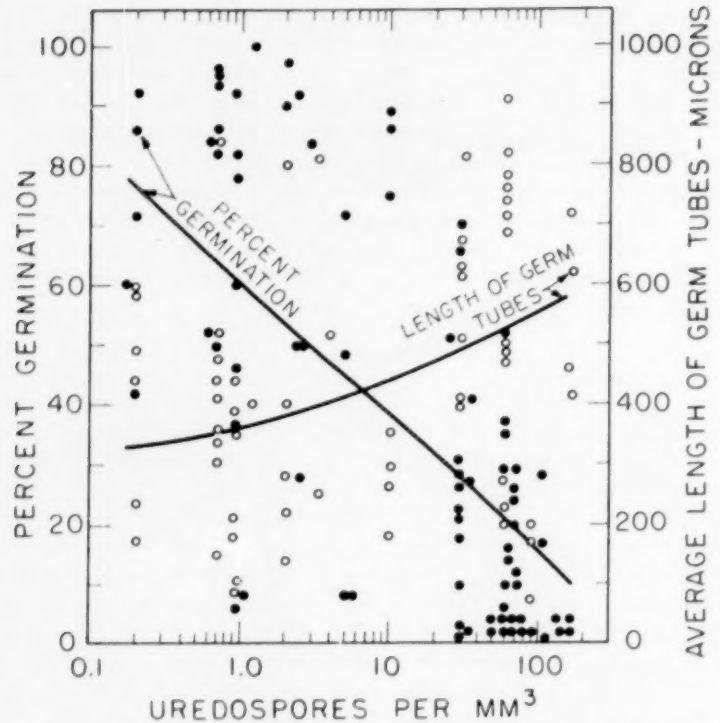

FIG. 1. Relation of spore dosage to percent germination and germ tube length of uredospores of *Uromyces phaseoli*.

Coumarin (Allen, **1955**), $KMnO_4$, and Tween 60, when added to the agar plates, greatly increased the percent germination of heavily seeded spores but did not raise the germination to the level of the thinly seeded spores.

For length of germ tubes the average deviation of a single determination from a mean of 10 determinations on the same plate was 34 percent as determined for 10 plates with germ tubes ranging from 160 to 1060 μ. For single treatments (plates) the maximum deviation of individual values was 50 percent and the minimum deviation was 19 percent. Significance of differences between treatments rests principally on the repetition of differences in independent experiments. In 12 trials on different days with a total of 36 pairs of plates of which one member of each pair was seeded lightly (3 or less spores per mm^3) and one member of each pair was seeded heavily (25 or more spores per

mm^3) the average length of germ tubes on the heavily seeded plates was greater than on the lightly seeded plates in 33 out of 36 pairs. On these same plates, the percentage germination on the lightly seeded members of the pairs was greater than on the heavily seeded members of the pairs in all cases.

In one trial the rate of growth of germ tubes was followed at intervals during the germination process at 25° C. The average rate of growth of lightly and heavily seeded spores (10 germ tubes measured for each value) was 19 and 21 μ per hour, respectively, at 0 to 4.5 hours after seeding, 62 and 74 μ per hour at 4.5 to 6.5 hours after seeding, 96 and 120 μ per hour at 6.5 to 7.5 hours after seeding, and 25 and 35 μ per hour at 7.5 to 10 hours after seeding. The previously recorded increase followed by a decrease in rate of growth of germ tubes of uredospores (Stock, **1931**) was therefore about equally exhibited by lightly and heavily seeded spores.

The effect of paraffin oil on self-stimulation and self-inhibition was tested in 4 trials. It was thought that the increased germination due to paraffin oil, discovered by Bailey (**1923**) with sunflower rust (*Puccinia helianthi*), might be due to the effect of the oil in absorbing the gaseous metabolites believed responsible for the reduction in percent germination. When 1 percent by volume of paraffin oil was added to the agar substrata on which the bean rust or sunflower rust spores were deposited, the percentage germination was greater than when no paraffin oil was added. When 10 ml of paraffin oil was added to the lids of inverted plates of uredospores on hardened agar, the percentage germination was not clearly greater than in plates without paraffin oil. Therefore while Bailey's finding was confirmed, it did not appear related to the self-inhibition phenomenon.

DISCUSSION

Because rust fungi live in such compatible associations with their hosts (Rice, **1927**) in comparison with other parasitic fungi, or for other reasons, it is commonly and perhaps logically assumed that they release fewer and/or less potent metabolites than do other fungi (Bessey, **1935**). While this may be true, studies presented here indicate that they release 2 powerful metabolites during the first few hours of germination. That the same metabolite which causes reduced germination can also increase the length of germ tubes is possible but unlikely. The metabolite causing self-inhibition of germination may inhibit the infection process (Yarwood, **1954**). Allen (**1955**) has shown that this inhibitor is effective at 0.5 ppm, which indicates that it is more toxic to spores than most fungicidal chemicals. No role or properties of the

self-stimulating factor other than stimulation is yet known. It may be one of these same metabolites which causes such a pronounced leaf roll of bean leaves within a few hours after inoculation (Yarwood and Cohen, **1951**). Further study of these metabolites might contribute to our knowledge of chemical control and acquired immunity with diseases caused by rust fungi.

SUMMARY

With increasing dosage of bean rust uredospores per unit area or per unit volume of agar substrata, the percent germination of the spores decreased but the length of germ tubes increased. This self-inhibition and self-stimulation of spore germination was greater at 25° C than at lower temperatures and greater in agar to which acid was added than where no acid was added, but was not greatly affected by paraffin oil. The self-stimulation of germ tube growth was apparent throughout most if not all the germination period.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

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PHYSIOLOGICAL STUDIES OF SOME FUNGI ASSOCIATED WITH POLE BLIGHT OF WESTERN WHITE PINE [1]

CHARLES D. LEAPHART

(WITH 1 FIGURE)

The study presented in this paper is one phase of a cooperative project directed toward the solution of pole blight of western white pine (*Pinus monticola* Dougl.). Pole blight is a disease of unknown cause, killing trees usually ranging in age from 40 to 80 years (**1**). Of the estimated 740,000 acres of pole-size timber in the western white pine type in the United States, 90,000 to 95,000 acres are now estimated to be moderately to severely affected (50 percent or more of the white pine stems per acre disease or killed) (**5**). Less intense damage probably occurs on an additional large acreage.

Many fungi have been isolated from blighted white pine, *Leptographium* sp. being one of the most common. Field inoculations made by personnel of the Division of Forest Disease Research during 1949 to 1951 on living white pine of various age classes with selected isolates of this fungus are as yet inconclusive (**3**). However, there is some evidence that *Leptographium* may play at least a secondary part in the pole-blight complex by causing lesions on both stem and roots. Field and laboratory tests [2] have also shown that some of the *Leptographium* isolates are extremely parasitic; others are less so; and still others are unable to parasitize living bark tissues.

Since there apparently are a number of different forms or varieties of this fungus, morphological and physiological studies on the ones asso-

[1] In partial fulfillment of requirements for the degree of Doctor of Philosophy, Yale University. The laboratory studies were conducted while the author was employed by the Division of Forest Pathology, U. S. Department of Agriculture.

The author received valuable guidance, cooperation, and advice from many individuals. Special appreciation is expressed to the following: Dr. L. M. Hutchins, Head Pathologist, and Dr. L. S. Gill, Principal Pathologist, Division of Forest Disease Research, U. S. Forest Service; Dr. J. S. Boyce, Professor, School of Forestry, Yale University; and Dr. V. M. Cutter, Jr., Professor, Department of Biology, University of North Carolina.

[2] Unpublished results of studies conducted by Lake S. Gill and the author.

ciated with western white pine were considered necessary. The studies reported in this paper are concerned with the physiological requirements of several forms. It was hoped that this work would provide an easy and accurate means of distinguishing the more pathogenic forms in addition to furnishing information on their physiology. If certain forms prove to be specific indicators of pole blight, a simple method for recognizing them as a group would be of primary importance in conjunction with future investigations of the disease.

The role that nutritional requirements play in the parasitic relationships between host and the fungus parasite is not clearly understood. Nor is it known why one host plant of a particular species is more susceptible than another. It is generally believed that the host plant must not be resistant to the attacking organism and further that the host must satisfactorily fulfill the nutritional requirements of the parasite. Whether or not the host supplies the latter requirements and more specifically in what proportions the concentrations of foods are present in it may help determine its resistance to the parasite.

An examination of the numerous reports in the literature to date on fungus physiology has failed to reveal similar work on the genus *Leptographium*. Although some immediate comparisons are available for the genus *Ceratocystis*, a species of which is included in the following study, generalization from the literature will not be considered here.

SOURCE OF TEST ORGANISMS

The organisms studied belong to the common group of fungi that cause both wilt and sap stain of many tree species. Some are well known because of their ability to parasitize and kill living trees, others because of their common occurrence as staining organisms, often resulting in serious degrade in lumber and other forest products.

With the exception of the *Ceratocystis* isolate, the fungi used in the study belong to the genus *Leptographium*.[3]

Since there appear to be several forms or varieties of *Leptographium* on white pine and on other coniferous species in western North America, a separate study would be required to distinguish between them. Therefore, no specific names have been assigned to these four isolates although morphologically they are somewhat similar to *L. lundbergii* Lag. &

[3] According to Goidanich (**4**), who undertook the problem of monographing this genus, it should be relegated to synonymy under the genus *Scopularia* as the imperfect stage of *Grosmannia*. While there has been some disagreement as to what generic name should be used, recent work (**13**) indicates that the genera *Scopularia* and *Hantzchia* should be considered as synonyms under *Leptographium*.

Melin. Further reference to them will be made by the stock culture number assigned the organism at the time of isolation. The source of each isolate is as follows:

No. *603–2*. *Leptographium* sp. Single spore transfer from original isolation. Isolated from blighted western white pine lateral root 3.1 feet from root collar. 7/2/48. Lower Benton Creek, Idaho.

No. *850*. *Leptographium* sp. Isolated from blue-stained area on stem of western white pine. 8/31/49. Slocan Lake, British Columbia.

No. *689–3*. *Leptographium* sp. Single spore transfer isolated from blue-stained area on a dead western white pine. 9/13/48. Jaypee Area, Idaho.

No. *1*. *Leptographium* sp. Isolated from blighted western white pine stem two feet above the root collar. 8/16/51. Nickleplate Mtn., Idaho.

No. *844*. *Ceratocystis pilifera* (Fr.) Moreau. Isolated from a blighted white pine tree felled in 1948. 8/11/49. Cedar Creek, Idaho.

The four *Leptographium* isolates show individual variations, both macroscopically and microscopically, when grown in culture. No. *689–3* can be distinguished from the others by its tight cork-screw-like mycelia, which are reddish-brown when young, becoming a dark brown when older. The mycelium of the other organisms is hyaline when young, black when old, and with the exception of No. *603–2* is fairly straight. The hyphae of the latter are somewhat cork-screw-like but very loosely so, being almost wavy in appearance. No. *689–3* is furthermore a very poor spore producer, conspicuously so in comparison with the others. Nos. *850*, *1*, and *603–2* all differ somewhat in conidia and conidiophore formation. On prune agar, No. *1* produces a variation of its aerial type conidiophore in profusion beneath the agar surface. This characteristic was not noted for Nos. *850* and *603–2* on the same medium. Although some submerged fruits of No. *603–2* were observed, they were similar in structure to the aerial type. No. *850* differs from No. *603–2* in the manner of conidiophore production as observed on various commercial media. The conidial clusters of the latter are generally borne on a simple septate conidiophore arising from the agar surface while they often are produced on a multiple-branched septate conidiophore in the case of No. *850*.

TEMPERATURE REQUIREMENTS

The selected isolates were grown under a series of temperature control tests to establish for each its optimum, maximum, and minimum insofar as was possible.

Materials and Methods. Each organism was tested in triplicate on four separate commercial agar media at nine different temperature levels, ranging from 0° to 40° C at 5° intervals. The 40° C tests were run only on malt extract agar. The media employed were Difco-Bacto products: corn meal agar, potato dextrose agar, prune agar and malt extract agar. Inoculum was obtained from stock cultures maintained in petri plates on corn meal agar. All series were grown in petri plates inoculated near one side by means of a special inoculating instrument.[4] Because of limited equipment, all temperature series were not started at the same time. However, the individual temperatures remained fairly constant and at no time during observations did they fluctuate more than about 2° C.

Results. Average daily growth of the organisms was recorded in millimeters as an average of 3 days growth succeeding a 3-day incubation period following inoculation. Recordings at 0° and 30° C were an average of 7 days growth so that enough growth had occurred to be measurable. These data were analyzed by an analysis of variance exclusive of the 40° C temperature results. By means of the "t-test" the most suitable temperature for growth for all isolates on all media was found to be 20° C. Therefore, a constant temperature of 20° C was chosen for subsequent studies in order to provide what appeared to be optimal conditions for further comparison between the organisms.

Of the temperature levels tested, the individual optimum is 25° C for No. *850* on all media combined, varying between 20° and 25° on the different ones; 20° for No. *603–2*, varying between 20° and 25°; between 15° and 20° for No. *689–3*; between 20° and 25° for No. *1*; and 25° for No. *844* on all the different media.

Though the minimum temperature for growth of the five isolates was not found, it approximates 0° C, the lowest temperature tested. For all organisms, growth gradually increases above 0° C up to their optimum after which there is a sharp reduction. No. *689–3* is the most sensitive to higher temperatures. Its maximum for growth is between 25° and 30° C, the latter being lethal after 7 days exposure. In general, 35° C for 7 days is lethal to No. *603–2*, while the others are able to live under this condition but do not remain viable after 3½ days at 40° C. The maximum temperature for No. *603–2* is between 25° and 30° C.

[4] This instrument was devised by J. W. Clark, Division of Forest Disease Research, U. S. Forest Service. The instrument consisted of a brass tube with a 1/16-inch bore fitted with a copper rod plunger. Since this tool produced uniform inoculum, it was used throughout all tests in this problem.

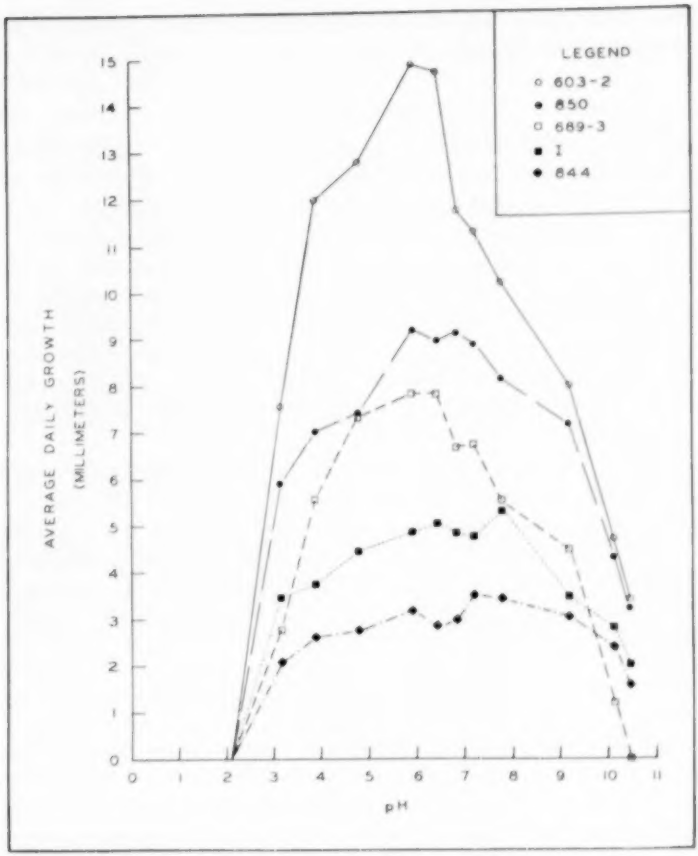

FIG. 1. Average daily growth of organisms on corn meal agar at various initial pH levels.

No growth occurs at 30° but cultures were viable after 7 days exposure. The maxima for Nos. *850, 1,* and *844* are between 30° and 35° C.

In his study of 6 isolates of *Ceratocystis pilifera* Lindgren (8) found that there were two distinct groups, one having approximate critical temperatures of 3°, 25° to 26°, and 31° to 34°, and the other approximately 2° to 4° C higher at the optimum and maximum levels. The isolate used in this study has somewhat lower minimum and higher maximum points with approximately the same optimum as the lower

temperature group. It is also able to remain viable at slightly higher temperatures than the ones he tested.

pH REQUIREMENTS

Materials and Methods. To establish the optimum pH for the five organisms, they were grown at a constant temperature of 20° C on corn meal and on malt extract agars at various pH levels, ranging from 2.1 to 10.45. Either prior to or after autoclaving, the media were adjusted with 1N solutions of potassium hydroxide or hydrochloric acid depending on the pH desired. Since rather large amounts of potassium hydroxide were needed to bring the level above pH 10.45, perhaps large enough to influence growth through the toxic effect of the potassium-ion, the maximum hydrogen-ion concentration at which the organisms would grow was not determined for any except No. *689–3,* which was at slightly above pH 10.0.

Results. FIG. 1 represents in graphic form the average daily growth of the isolates on corn meal agar adjusted to various pH levels. A statistical test showed that, for all isolates, growth at pH 5.9 to 6.4 was best (significant at the 1 percent level). Therefore, to minimize any differential acidity influence upon growth, all media in the subsequent vitamin, carbohydrate, and nitrogen tests were adjusted to approximately pH 6.0.

VITAMIN REQUIREMENTS

Various workers (**10, 11, 14, 15**) have demonstrated that a number of fungi are heterotrophic with respect to certain vitamins and vitamin-like substances. The purpose of the following experiment was to determine the vitamin deficiencies of the five isolates. Among the organisms tested, two types of heterotrophy or vitamin deficiency were noted; namely, (1) complete and (2) partial. While certain partial deficiencies were noted in this test, no attempt was made to determine what type of deficiency these were or whether they might be conditioned ones, i.e., ones that are influenced by variations in pH, temperature, and media composition.

Materials and Methods. To test growth-factor requirements, the basal nutrient solution was prepared as follows: The following compounds were added to one liter of double distilled water: $CaCl_2 \cdot 2H_2O$, 0.33 gm; KH_2PO_4, 1 gm; NH_4NO_3, 2 gm; $MgSO_4$, 0.25 gm; glucose, 30.0 gm. Trace elements were added, expressed as parts per million, to this medium as follows: Zn, 0.1; Mn, 0.01; Fe, 0.08; Mo, 0.01; Cu,

0.02; and B, 0.1. Nine vitamins were employed in the quantities, expressed in micrograms per liter, as follows: i-inositol, 1000; thiamine hydrochloride, 1000; calcium pantothenate, 200; riboflavin, 200; pyridoxine hydrochloride, 200; p-aminobenzoic acid, 200; nicotinic acid, 400; folic acid, 0.5; and biotin, 0.5.[5] Thirteen variations of the basic media, constituting a series, were prepared as follows: 9 each complete in all but one of the 9 vitamins used, 1 with no vitamins, 1 with all vitamins, 1 with 5 grams bacto-peptone added, and 1 with peptone plus thiamine added.

All glassware used in the experiment was chemically cleaned with a chromic-sulphuric acid mixture and was washed first with distilled, then hot tap, and finally distilled water. The media were dispensed into 250 ml Erlenmeyer flasks in 50 ml quantities. The flasks were then plugged with cotton plugs wrapped in gauze and autoclaved at 15 lbs pressure for 30 minutes. After autoclaving, the culture solutions were adjusted to approximately pH 6.0 with 1N NaOH; about 0.05 cc NaOH per flask was required to bring the solution to the desired pH level.

Preliminary tests showed that growth sometimes occurred on the basal media solidified with washed agar and devoid of vitamins, but was insufficient to provide inoculum for a complete series. Inoculum for all four series was, therefore, obtained from cultures grown on the solid basal medium supplemented with all nine vitamins but lacking in a sugar source. A spore suspension type of inoculum might have served this purpose much more adequately, except that No. *689–3* does not produce enough spores to provide inoculum comparable to the other isolates.

Each organism was tested in triplicate at a temperature of 20° C. Growth was measured at the end of a 15-day period. Every second day the flasks were shaken to aerate the cultures. At the end of the period the amount of growth was determined by taking the oven-dry weight of the mycelium after it had been autoclaved, filtered, and washed. Filtering was accomplished by passing the autoclaved mycelium and medium through Armstrong's white filter paper. The filtrate was set aside to determine pH. The mycelium and filter paper were washed with distilled water three times prior to drying.

Results. Growth of the isolates is shown in TABLE I and is represented by an averaged weight of the mycelium from a triplicate set of

[5] With the exception of riboflavin and thiamine hydrochloride which were purchased from the Eastman Kodak Company, the other vitamins were General Biochemicals, Inc., products. The glucose was obtained from Eastman.

flasks per organism per medium. All five are completely heterotrophic with respect to biotin, and No. *844* with respect to pyridoxine. With these exceptions and possibly that of No. *1* which appears to require thiamine for good growth, no complete heterotrophisms were exhibited by the test fungi. While partial pyridoxine and thiamine deficiencies have been interpreted as existing for some of the organisms, the reasons for this interpretation may appear rather vague. It should, therefore,

TABLE I

GROWTH RESPONSE ON MEDIA DEVOID OF CERTAIN VITAMINS

Vitamin omitted from medium	Organism				
	603–2	850	689–3	1	844
All	0.0[1]	0.0	0.0	0.0	Trace
Biotin	0.0	0.0	Trace	0.0	6.1
Pyridoxine	50.1	33.1	17.8	33.6	3.6
Ca Pantothenate	48.5	42.1	21.1	36.8	39.5
Folic acid	62.0	48.7	19.2	32.7	38.7
Nicotinic acid	55.9	43.1	18.7	36.0	37.9
PABA	48.8	42.5	20.2	33.7	38.4
Inositol	48.2	38.7	20.8	34.6	38.7
Riboflavin	55.6	44.1	19.2	39.1	45.6
None	64.3	47.4	24.9	39.7	37.2
Thiamine	34.0	22.8	17.3	7.6	37.5
Peptone+Thiamine[2]	67.3	67.1	41.8	54.5	42.0
Peptone[3]	44.3	32.7	44.1	14.7	35.2

[1] Growth is represented by the averaged weight of mycelium in milligrams from a triplicate set of flasks per organism per medium after period of 15 days at 20° C.
[2] Only 5 grams of peptone plus 1000 micrograms of thiamine added per liter of basal solution.
[3] Only 5 grams of peptone added per liter of basal solution.

be pointed out that the degree of partial deficiency may vary widely, from slight to nearly total (8, p. 177). However, the complete deficiencies observed in No. *844* are based on what is judged insufficient growth to have been grouped in the partial category.

According to an analysis of variance of the growth data, certain real differences occurred in the growth of the organisms in various media. The analysis with regard to Nos. *603–2* and *850* showed that significantly more growth occurred on the thiamine-deficient medium than on the medium devoid of vitamins but significantly less than on the media with no deficiency. A partial thiamine deficiency was, therefore, recorded for both of these organisms. Although some growth takes place in the medium from which thiamine had been omitted in the case of isolate No. *1*, the response was not considered strong enough to place this organism in the partially deficient category for this vitamin. There-

fore, it was listed as probably completely deficient. Growth responses on peptone and peptone-plus-thiamine in liquid cultures also point to heterotrophism with respect to thiamine where this occurs. A typical analysis of Bactopeptone obtained from the Difco Laboratories shows the following vitamins to be present in micrograms per gram of peptone: pyridoxine, 2.50; biotin, 0.32; thiamine, 0.50; nicotinic acid, 35.0; and riboflavin, 4.0. Since only 5 grams of peptone were added per liter of medium, the amount of thiamine alone in the peptone medium was not sufficient to support satisfactory growth for No. *1* in which the complete deficiency probably occurs. The partial thiamine deficiencies in Nos. *603–2* and *850* on these media are not so clearly defined.

Since growth response of No. *850* in pyridoxine-deficient medium was below average, a partial deficiency is indicated for this organism. This is also supported by the statistical analysis. With the exceptions just mentioned, no vitamin deficiencies were found. The organisms are completely autotrophic with respect to these vitamins or do not require them for growth.

CARBOHYDRATE UTILIZATION

While a fungus utilizes a carbohydrate through a series of transformations, no attempt was made in this study to determine what the steps in the reduction might be but rather to show which ones of the carbon compounds tested are the most suitable for growth in terms of production of mycelium.

Materials and Methods. The basal solution used to test carbon utilization was as follows: To the basal solution of the vitamin requirement study, all vitamins except riboflavin were added on the basis also described in that experiment; however, the carbohydrate source was deleted from the solution. All water used in this study was double distilled. The carbon compounds were tested on a single additive basis and were added in an amount equivalent to 12.0 grams of carbon per liter of basal solution. The carbohydrates employed were l(+)-arabinose, d(+)-cellobiose, glucose, d(+)-galactose, d(−)-levulose, d(+)-maltose, d(+)-mannose, mannitol, d(+)-melezitose, d(+)-melibiose, raffinose hydrate, l(−)-sorbose, soluble starch, sucrose, and d-xylose.

Each organism was tested in triplicate on all media. Sixteen variations in media were prepared, one with each of the 15 compounds and one with no carbohydrate. These were dispensed in 50 ml quantities into 250 ml flasks. With the exception of maltose, sucrose, cellobiose, melibiose, melezitose, and raffinose, all the compounds were added to

the basal solution prior to dispensing and autoclaving. These seven, however, were dissolved in water, cold sterilized by means of a Seitz filter, and were added in appropriate amounts to sterile flasks containing only the basal solution. All flasks were then adjusted to approximately pH 6.0 with 1N NaOH.

The inoculum was obtained from stock cultures grown on basal medium without a source of carbon but solidified with washed agar. Any carbohydrate contamination carried into the medium by the inoculum could, therefore, only be obtained by the organisms from the agar.

TABLE II

EFFECT OF CARBOHYDRATE SOURCE ON GROWTH AFTER 15 DAYS AT 20° C.

Carbohydrate added to media	Organism				
	603–2	850	689–3	1	844
Glucose	79.8	45.5	24.5	43.6	35.3
d(−)-Levulose	80.9	24.1	19.6	30.9	40.8
d(+)-Galactose	38.0	32.1	17.7	21.7	38.0
d-Xylose	32.8	24.2	7.5	15.8	15.4
d(+)-Mannose	94.4	37.1	17.7	41.4	44.0
l(+)-Arabinose	21.1	16.2	3.4	15.9	27.5
d(−)-Sorbose	3.5	Trace	1.3	1.0	23.0
d(+)-Maltose	41.1	20.9	13.4	14.6	29.7
d(+)-Cellobiose	25.4	16.3	9.4	17.8	36.9
d(+)-Melibiose	Trace[1]	Trace	Trace	Trace	Trace
Sucrose	Trace	Trace	Trace	Trace	Trace
d(+)-Melezitose	1.5	1.5	Trace	2.5	28.0
Raffinose hydrate	1.8	2.5	1.6	1.6	27.1
Starch (soluble)[2]	84.5	43.8	32.2	32.5	39.1
Mannitol	4.0	2.6	1.1	11.0	8.8
No carbohydrate	Trace	Trace	Trace	Trace	Trace

Growth measurements are an average of the oven-dry mycelium weights, expressed in milligrams, from 3 flasks.

[1] Average weight did not exceed 1.0 milligram.

[2] Autoclaved prior to testing.

Growth was allowed to continue for 15 days at 20° C during which time the cultures were aerated by shaking once every two days. The amount of growth on the various media was determined by obtaining the oven-dry weights of the mycelium in the same manner as previously explained in testing for vitamin requirements.

Results. The measure of carbohydrate utilization in this test is the amount of mycelium, oven-dry weight, produced by the test organisms. TABLE II represents the averaged weight of mycelium of a triplicate set per medium per organism. In all cases some mycelium, at least, was produced but, in those where trace amounts are indicated, the mycelium did not weigh more than one milligram. Trace amounts of growth were

probably due to carbon contamination from the inoculum. Sufficient carbon was carried into the medium by the inoculum to initiate growth; but, since the carbohydrate in the medium could not be utilized by the fungus, no further growth occurred.

Sucrose and d(+)-melibiose were considered poor sources of carbon for all five organisms. Raffinose hydrate, l(−)-sorbose, and d(+)-melezitose were not utilized except by No. *844* for which they were found to be fair to good sources. Mannitol, a sugar alcohol, was not used efficiently by any of the test fungi. Dextrose, d(−)-levulose, d(+)-mannose, and soluble starch are, in general, good sources of carbon. Since the starch was autoclaved prior to use, it is probable that hydrolysis of starch occurred in the culture solution; therefore, results quite similar to those on dextrose were obtained. Utilization of the remaining carbohydrates ranked intermediate between good and poor although some appeared to be more efficiently used than others.

NITROGEN UTILIZATION

According to their ability to utilize the various sources of nitrogen, fungi have been classified by Robbins (**9**, pp. 243–244) into four groups as follows: (1) nitrogen-fixing, (2) nitrate-ammonium, (3) ammonium, and (4) organic nitrogen organisms. The purpose of the following test is to point out which forms of nitrogen can be utilized as sole sources by the fungi studied. However, it was not possible in this study to test their ability to utilize gaseous nitrogen.

Materials and Methods. The technique and procedure for testing the utilization of various nitrogen compounds was similar to that employed for carbohydrates. The basal medium was the same except that it contained 30.0 grams of dextrose per liter of basal solution and the nitrogen source was deleted. The nitrogen sources were tested individually as follows: an amount equivalent to 157 milligrams of nitrogen was added per liter of basal solution prior to autoclaving. Twenty-six variations in media were employed, one with each of the 25 nitrogen compounds listed in TABLE III and one with no nitrogen. The latter served as the control.

Inoculum was obtained from stock cultures maintained on the complete basal medium solidified with washed agar but with the nitrogen source omitted. Growth occurred on this type of medium presumably because there is a trace of nitrogen in the agar. According to Leonian and Lilly (**6**, p. 536), this trace amount may be sufficient to give fungi a start in utilization of nitrogenous compounds not normally used.

TABLE III

EFFECT OF NITROGEN SOURCE ON AMOUNT OF GROWTH AFTER 15 DAYS AT 20° C

Nitrogen added to media	Organism				
	603-2	850	689-3	1	844
Organic Compounds					
dl-Alpha alanine	34.9	12.9	11.2	22.8	41.1
l(+)-Arginine monohydrochloride	72.4	45.4	26.4	51.4	40.2
l-Asparagine	93.2	59.7	22.8	49.2	57.5
l-Aspartic acid	73.2	38.3	11.1	48.8	32.8
Cysteine hydrochloride	Trace[1]	Trace	4.7	Trace	4.4
Glycine	11.9	3.8	11.3	5.5	10.4
l(+)-Histidine monohydrochloride	52.0	29.1	4.8	34.2	13.5
dl-Isoleucine	12.0	11.8	5.7	12.3	21.0
l(−)-Leucine	32.9	27.3	14.9	21.5	24.2
l(+)-Lysine monohydrochloride	5.4	7.5	Trace	Trace	Trace
dl-Methionine	19.0	16.4	8.2	16.5	23.6
dl-Phenylalanine	14.1	14.2	5.8	15.2	16.3
l(−)-Proline	46.7	19.2	18.2	27.8	38.6
dl-Serine	64.9	27.3	15.1	35.5	26.5
dl-Threonine	14.5	7.0	6.8	11.1	24.0
l(−)-Tryptophane	11.0	17.8	2.7	10.6	21.0
dl-Valine	12.8	17.1	5.1	26.5	24.4
Urea	63.7	35.5	21.7	19.5	45.4
Inorganic Compounds					
Sodium nitrite	00.0	00.0	00.0	00.0	Trace
Potassium nitrite	00.0	00.0	00.0	00.0	Trace
Potassium nitrate	85.4	20.0	14.4	24.6	Trace
Magnesium nitrate	83.4	15.4	11.1	16.1	Trace
Sodium nitrate	61.6	13.4	10.0	16.1	Trace
Ammonium nitrate	57.6	23.1	12.8	21.2	38.7
Ammonium sulfate	51.1	40.7	15.7	24.7	38.7
No Nitrogen	Trace	Trace	Trace	Trace	Trace

Growth measurements are an average of the oven-dry mycelium weights, expressed in milligrams, from 3 flasks.

[1] Average weight did not exceed 1.0 milligrams.

Despite this possibility, this type of inoculum was considered the most suitable, with the facilities available and under the test conditions.

Results. TABLE III shows the amount of growth, oven-dry weight of mycelium, made with the respective compounds as nitrogen sources by the five isolates. The medium to which no nitrogen was added and which served as control in the experiment supported trace amounts of growth of the five isolates. As indicated previously, this is attributed to a nitrogen carry-over in the inoculum. However, since no additional nitrogen source was available, the fungi ceased to grow after producing

limited growth (TABLE III). From this, one might presume that they are not able to assimilate gaseous nitrogen, but the test conditions were not considered adequate proof.

Of the inorganic sources employed, nitrite-nitrogen could not be utilized effectively by any of the organisms. In fact, there appeared to be some unknown toxic condition existing in liquid culture which completely inhibited growth in the case of the *Leptographium* isolates although No. *844* did produce a trace amount of growth. According to Foster (2, p. 491), nitrites are not utilizable when the medium is highly acid, in which case the KNO_2 is toxic. The limiting factor of growth could not be attributed to hydrogen-ion concentration. In the case of the *Leptographium* isolates the medium was pH 6.2 prior to inoculation and pH 5.7 after the test run was completed and the cultures were autoclaved well within the favorable pH range of the organisms.

With the exception of organism No. *844*, all could use nitrate-nitrogen but only No. *603–2* could utilize it as well as some of the better organic sources and more efficiently than the ammonium-nitrogen sources. Organisms Nos. *850* and *844* grew nearly as well on the ammonium form as on the more utilizable organic forms; it was only a fair source of nitrogen for Nos. *603–2*, *689–3*, and *1*.

None of the organic nitrogen sources employed in this study were considered essential in the sense that any were required for growth, provided a satisfactory inorganic compound was available. Asparagine, which is generally one of the most utilizable nitrogen sources for fungi (2, p. 502), was the best for all the organisms except No. *689–3* for which arginine was found to be the most suitable. Urea was a fair to good source for all except No. *1* which showed less than average growth in media containing this compound.

Cysteine was the only organic source tested which may be toxic to several of the fungi. In two flasks out of three with organisms Nos. *850* and *1* and one of three with No. *603–2* no growth occurred while trace amounts of growth were present in the remaining flasks. Whether or not this amino acid is actually toxic could only be determined by more detailed tests, particularly with regard to the concentration of the compound in the medium and to the reaction of the various fungi in media containing other nitrogen sources in addition to cysteine.

DISCUSSION

The results of most physiological studies of fungi do not appear to have immediate practical applications to control measures under forest conditions. However, as our knowledge of host requirements and pre-

ferred ecological associations increases, the adaptability and growth of a parasite in the host environment can better be understood and evaluated by its physiological requirements. The present studies determine some of the prerequisites for growth of the five isolates under investigation. It should be recognized that additional work is desirable to define optimal and inhibitory levels of various nutrient, acidity, and temperature combinations. This, combined with additional host-fungus relationship studies, might explain why some *Leptographium* isolates are selective in parasitizing, to a limited extent, western white pine and not certain other closely associated tree species. Furthermore, an explanation of why certain individual white pine trees and not others are parasitized by this fungus may be revealed.

A comparison of the nutritional requirements of these organisms with those of filamentous fungi in general does not reveal any extraordinary variations. The vitamin deficiencies encountered in this study are the ones generally occurring in fungi. The results, nevertheless, are of interest because of the different requirements of the *Leptographium* isolates. All five organisms require biotin for growth. Pyridoxine is required by No. *844* and thiamine by No. *1*. A partial thiamine deficiency in No. *603–2* and partial deficiencies of both pyridoxine and thiamine in No. *850* were observed. With the exception of biotin, No. *689–3* has no deficiencies.

Robbins and Ma (**12**, p. 841) reported a complete deficiency of biotin and questionable partial deficiencies of both thiamine and pyridoxine in an isolate of *C. pilifera* from lumber in Louisiana. In a table of this same report (**12**, p. 842), however, they reported a complete pyridoxine, partial (?) thiamine, and no biotin deficiency in the same organism. Presumably an error was made in the table because the deficiencies listed on page 841 in their report more closely correspond with the ones noted in No. *844* used in this study. Nevertheless, the difference in thiamine requirements of the two isolates suggests an intraspecific variation.

Utilization experiments with 15 carbohydrates and 25 nitrogen compounds indicate some diversity in the capabilities of the organisms to use the various compounds for growth. The *Leptographium* isolates apparently have similar enzymatic potentials in being able to utilize certain carbon and nitrogen compounds but differ in this respect from *C. pilifera*. The inability of all five isolates to utilize sucrose is of interest but this is not exceptional except for the *Ceratocystis* isolate. Since this fungus alone can utilize raffinose, it should be able to use all five sugars, sucrose, melibiose, glucose, galactose, and levulose, which

are the normal hydrolytic products of raffinose. Apparently raffinose is split by the organism in a different manner than above and melibiose and levulose are formed upon hydrolysis. The levulose fraction is assimilated and the melibiose fraction accumulates since this organism does not produce the necessary enzyme to split it.

Study of the literature concerning physiological and nutritional requirements of fungi indicates that dependence on established requirements as criteria for differentiating between various isolates or strains of a single fungus species may lead to erroneous conclusions. Some that appear morphologically similar will exhibit extreme differences and others will be intermediate between the extremes (2, p. 186). This would suggest that it may be impossible to differentiate by physiological behavior between apparently similar forms of *Leptographium*. There is still some reason to believe that parasitic forms of this species may be distinguished from the non-parasitic ones. The initial and principal phase of work has been completed by determining general growth conditions and nutritional requirements of the species. Mass testing of a large number of isolates of which the parasitic potential has already been established should provide a pattern by which isolates can be separated on their parasitic capabilities. Of the *Leptographium* isolates used in this study, No. *689–3* is unable to parasitize living cambium, Nos. *603–2* and *850* are able to do so, but the parasitizing potential of No. *1* is unknown.[6] With this small number of isolates, establishment of adequate pattern limits was not feasible.

The evidence that the non-parasitic form (No. *689–3*) of *Leptographium* differs physiologically is demonstrated principally by its sensitivity to high temperatures and its difference in vitamin requirements. Its probable resistance to the inhibitory effect of cysteine on growth and its reaction to arabinose are of doubtful value. Irrespective of inherent growth rates, one isolate of this fungus species appeared able to utilize a certain carbohydrate or nitrogen compound as poorly or as well as the other three. Certain clear-cut differences in carbon and nitrogen utilization and in other growth requirements have been pointed out between the *Ceratocystis* isolate and the *Leptographium* ones. Where differences do occur between isolates of the latter genus, they are so negligible that differentiation by this means does not appear reliable. Repeated tests on what seem to be key compounds might clarify the apparently insignificant differences.

It is concluded that differentiation between the isolate of *C. pilifera* and the *Leptographium* isolates used in this study may be possible, based

[6] Unpublished results of a study by the author.

on their respective abilities or inabilities to use certain carbon or nitrogen compounds. This basis would not appear to be of practical use in distinguishing between isolates of *Leptographium;* however, certain trends in possible differentiation were observed and would merit further testing.

DIVISION OF FOREST DISEASE RESEARCH,
INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION,
U. S. FOREST SERVICE,
OGDEN, UTAH

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THE ASSOCIATION OF YEASTS WITH CERTAIN BARK BEETLES

M. Shifrine and H. J. Phaff

There is now a vast literature describing various aspects of the relationship between insects and microorganisms (see *e.g.* Steinhaus, **1946**). Taxonomic studies on yeasts associated with bark beetles began when Beck (**1922**) described a new species of yeast, *Endomyces bisporus,* which she isolated repeatedly from the galleries of *Ips typographus* attacking spruce in Austria. Siemaszko (**1939**) also noted this yeast associated with *Ips typographus* in *Picea excelsa* in Poland. Stelling-Dekker (**1931**) renamed this yeast *Endomycopsis bispora,* because it forms blastospores besides true mycelium. It was finally renamed *Hansenula beckii* (Beck) by Wickerham (**1951**) since it utilizes nitrate-ion as a single source of nitrogen, a typical characteristic for species of *Hansenula.* Lodder and van Rij (**1952**), however, feel that the yeast should be maintained in *Endomycopsis* in spite of the fact that it is nitrate positive, because of its abundant production of true mycelium. It may be well to point out that all studies on this yeast were done with the original isolate of Beck. The only other report in the literature, which describes the occurrence of this yeast in *Ips typographus,* is a paper by Grosmann (**1930**). Her insect material was collected from two well separated areas in central Germany. Beside *E. bisporus,* she found various wood staining fungi and another yeast producing hat-shaped spores (2–4 per ascus) which dehisce early. This yeast did not produce mycelium or pseudomycelium. The possibility was considered that this yeast might belong to *Hansenula.* A third type did not produce spores. Grosmann also found yeasts in the intestinal tract of adults and larvae and in fresh excreta. The view was expressed that the yeasts are not essential for the growth of the insects, but merely live in association with them as commensals. Rumbold (**1931**) and Leach, Orr and Christensen (**1934**) also noted the regular occurrence of yeasts and yeast-like organisms in association with bark beetles and blue-staining fungi. Somewhat later Holst (**1936**) showed that a new species of yeast, which was named *Zygosaccharomyces pini,* could be isolated regularly from phloem galleries and the bark beetles *Dendroctonus* and *Ips* from various parts of the United States. This yeast, lacking

hyphae and pseudohyphae, forms hat-shaped spores, which is not common in the genus *Zygosaccharomyces*. Rumbold (**1941**) studied the fungi carried by certain bark beetles and confirmed the presence of *Z. pini*. In addition several species of *Candida* (termed *Monilia* in her paper) were found in two species of *Dendroctonus*. The yeasts appeared to promote the growth of *Ceratostomella* fungi on wood. Lodder and van Rij (**1952**) considered *Z. pini* Holst synonymous with *Z. pastori* Guilliermond. However, Wickerham (**1952**), by using a larger number of carbon assimilation reactions, found seven compounds which *Z. pini* can assimilate, but which *Z. pastori* cannot. He therefore considers *Z. pini* Holst a distinct and valid species.

It can be seen from the foregoing review that information on the yeast flora associated with bark beetles is extremely limited and fragmentary. Moreover, the taxonomic procedures used in the older work have been revised considerably in recent years. The purpose of the present paper is to discuss the yeasts associated internally with various species of the bark beetle genera *Dentroctonus* and *Ips*, collected from different species of trees.

EXPERIMENTAL METHODS

Collection of the insect material: The majority of the material was collected in Lassen and El Dorado Counties of Northern California (August 1952 and July 1953). An occasional sample was taken from Crystal Bay (Lake Tahoe). Sections of infested trees or bark containing brood material were brought to the laboratory. It was found undesirable to remove beetles and larvae from the trees in the forest, since the intestinal content appears to be digested rather rapidly at room temperature. This finding is quite in line with similar observations on *Drosophila* (Shehata and Mrak, **1952**) and *Carpophilus* beetles (Miller and Mrak, **1951**). Occasionally, if there was a delay between removal of the material from the tree and the time of dissection, the insects were stored in the refrigerator.

Dissection procedure: The procedure of dissection was adapted after the one described by Miller and Mrak (**1951**). In order to avoid contamination with yeasts and molds accidentally present on the exterior of the insects, all isolations were made by streaking only the internal parts of adults, larvae, pupa and callow adults. It was found that dipping of the insects for 5 minutes in a 0.5 per cent solution of $HgCl_2$ killed all the microorganisms adhering to the surface. After thorough rinsing in sterile water the adults were placed, dorsal side up, on a sterile paraffine block, by melting a small area of paraffine and allowing it to harden

after placing the beetle in the melted wax. After removal of the elytra and wings with dissecting needles the head was severed and a longitudinal cut was made from the prothorax, through the metathorax and abdomen, without severing the gut. A scalpel made of a triangular piece of razor blade attached to a glass rod was used for this purpose. The cut was then widened with the aid of two needles and the alimentary canal was pulled out and placed on a wort agar plate, where it was pulled apart with the needles. Finally it was streaked with a loopful of sterile water over the surface of the plate. A pupa or larva was dissected by placing it on a sterile slide, under a wide field microscope, and pulling it apart with two needles. The aqueous suspension was then streaked.

Isolation of the yeasts: Yeasts and bacteria always appeared first, molds only after about one week of incubation. The yeast colonies which came up (the number varied from a few to about 300) were inspected macroscopically by means of a wide field microscope and those which showed differences were brought in pure culture by conventional streaking techniques. Often only one type of colony appeared on a plate, sometimes two and rarely three kinds. The colonies which were picked were also inspected microscopically since ascospores frequently occur on the isolation medium.

Identification of the yeasts: Taxonomic procedures employed were those of Lodder and van Rij (**1952**) and for *Hansenula* those of Wickerham (**1951**) except that the assimilation of carbon compounds was studied in part by the replica plating procedure of Shifrine, Phaff and Demain (**1954**). Because of the limited number of physiological reactions used in the system of Lodder and van Rij, it is frequently found that several species of yeast with distinctly different biochemical properties are grouped together in one species. This disadvantage is greatly reduced in the system of Wickerham, but unfortunately it is as yet available only for species belonging to *Hansenula*. The procedure followed by us was to attempt classification of the yeasts not belonging to *Hansenula* by the Dutch system and whenever this proved impossible, the isolates were described as new species. In studying the properties of the new species we have tested the assimilation of most of the carbon compounds suggested by Wickerham (**1951**). In order to save space only the positive results are recorded. This necessitates a listing of all the compounds used for the assimilatory studies. They are the following: D-glucose, D-galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, i-

erythritol, adonitol, dulcitol, D-mannitol, D-sorbitol, alpha-methyl-D-glucoside, salicin, gluconic acid, calcium 2-keto-D-gluconate, potassium 5-keto-D-gluconate, DL-lactic acid, succinic acid, citric acid and i-inositol.

RESULTS

Taxonomy of the yeasts: One hundred and sixty-nine isolates were obtained and identified. They are listed in TABLE I. The sporulating yeasts (72 isolates) fell into the genera *Hansenula, Saccharomyces* and *Pichia.*

TABLE I

SPECIES OF YEAST ASSOCIATED WITH THE BARK BEETLES IPS AND DENDROCTONUS

Yeast	No. of isolates
Saccharomyces pini (Holst) nov. comb. 1936	32
Hansenula capsulata Wickerham 1951	36
*Pichia haplophila**	3
*Candida silvicola**	70
C. curvata (Diddens et Lodder) Lodder et van Rij 1942	15
C. parapsilosis (Ashf.) Langeron et Talice 1928	2
C. mycoderma (Reess) Lodder et van Rij 1952	3
C. rugosa (Anderson) Diddens et Lodder 1917	1
*Torulopsis nitratophila**	1
*T. melibiosum**	2
*Rhodotorula crocea**	1
Cryptococcus diffluens (Zach) Lodder et van Rij 1934	2
Cr. neoformans (Sanfelice) Vuillemin 1894	1
Total number of isolates	169

* Described as new in present paper.

A. HANSENULA: Thirty-six isolates were found to be similar to *H. capsulata.* Our isolates of *H. capsulata* fit the description of the species given by Wickerham (**1951**) except for two of the strains, which failed to utilize alpha-methylglucoside. This was tested both by the auxanographic technique and the replica plating method. Rhamnose was utilized by only 18 of the isolates. These compounds were reported by Wickerham to be assimilated sometimes weakly.

B. SACCHAROMYCES: Thirty-two isolates were found to be similar to *Zygosaccharomyces pini* Holst (**1936**). This species was placed in synonymy with *Zygosaccharomyces pastori* Guilliermond by Lodder and Kreger van Rij. However, Wickerham (**1952**), on the basis of more extensive carbon assimilation reactions, has expressed the view that the two yeasts are distinctly different species. On the basis of information regarding the carbon assimilation patterns of the two authentic species

kindly made available to us by Dr. Wickerham, our isolates were clearly identical with *Zygosaccharomyces pini*. Examples of compounds assimilated by *Z. pini* but not by *Z. pastori* are cellobiose, D-ribose, i-erythritol, adonitol and salicin. In view of the proposed elimination of *Zygosaccharomyces* as a genus or subgenus by Lodder and van Rij, with whom we concur, the isolates should be named **Saccharomyces pini** (Holst) comb. nov. We should like to point out that the inclusion of poorly fermentative yeasts (glucose fermented weakly and the other sugars not at all) with distinctly hat-shaped spores in *Saccharomyces* seems rather illogical. If the organisms produced a pellicle on malt extract and formed a pseudomycelium they would fall into the genus *Pichia*. The same situation existed in *Hansenula* until Wickerham (**1951**) showed that there were species belonging to this genus without pseudomycelium and film-forming ability. On similar grounds it may be desirable to modify the definition of *Pichia* to include non-film-forming and non-pseudomycelium-forming yeasts with hat-shaped spores. If this were done *S. pini* could be transferred to *Pichia*.

C. PICHIA: Three similar isolates were obtained belonging to this genus. Two cultures were isolated from one individual of *Dendroctonus jeffreyi* and the third from *Ips emarginatus*, both in jeffrey pines. Because of the absence of fermentation, the presence of a distinct and dull film on liquid wort and alcohol medium and the formation of hat-shaped spores with a short brim, the organisms belong in the genus *Pichia*. The isolates have, however, the unusual characteristic of not producing a pseudomycelium or the long cylindrical cells associated with a pseudomycelium. In this respect the isolates form an intermediate position between *S. pini* and a typical representative of *Pichia* (*cf.* discussion of the *S. pini* isolates). Based on these characteristics and the assimilation of both glucose and galactose, the organisms form a new species. Since the vegetative cells conjugate almost invariably prior to sporulation we propose the name *Pichia haplophila*. Culture *52-11* has been designated as the type.

Pichia haplophila sp. nov.

Conjugatio heterogamica. Ascosporae petasiformes, 2 ad 4 in quoque asco. Cellulae vegetativae rotundae ad ellipsoideae, 1.8–2.5 × 2.5–5.0 μ. Pellicula formatur super media liquida. Pseudomycelia at vera mycelia deficiunt. Glucosum non fermentatur. Glucosum, galactosum, erythritolum, adonitolum, dulcitolum, mannitolum, et sorbitolum assimilantur, at non nitras kalicus, sucrosum, maltosum, lactosum et raffinosum. In medio cum alcohole aethylico crescit. Arbutinum non finditur.

Growth in malt extract: after 24 hours, cells globose to oval, few elongate cells present, 1.8–2.5 × 2.5–5.0 μ, single or in clusters. Complete thin dull smooth pellicle. After 3 weeks complete film and heavy sediment. Streak cultures on malt agar: after 3 weeks at 25° C greyish to cream colored, smooth, dull, pasty, cross-section convex rather spreading, border entire. Slide cultures on potato agar dextrose: no pseudomycelium formation. Sporulation: spores formed easily on wort agar after heterogamic conjugation. Asci rupture readily after maturation, releasing 2 to 4 hat-shaped spores which have a rather short brim. Spores may clump together in masses. Fermentation: absent. Carbon compounds assimilated: glucose, galactose, D-xylose, L-arabinose, D-arabinose (latent), D-ribose, ethanol (with the formation of a thin, dull, white film), glycerol, i-erythritol, adonitol, dulcitol, mannitol, sorbitol and Ca-2-ketogluconate. The other carbon compounds are not used. Added vitamins are required in synthetic media. Assimilation of nitrate: absent. Splitting of arbutin: absent.

The non-sporulating yeasts belong to the genera *Candida, Torulopsis, Rhodotorula* and *Cryptococcus.*

D. CANDIDA: Ninety-one isolates were placed in this genus. Of this group, 70 could not be identified with any known species and they are here described as *C. silvicola* because of their common occurrence in the forest. Of the others, 15 were similar to *C. curvata,* 2 to *C. parapsilosis,* 3 to *Candida mycoderma* and 1 to *C. rugosa.*

Candida silvicola appears to be the most common yeast isolated during our survey. Sixty-nine of the isolates had a similar assimilation pattern and were also otherwise identical, except that the streak cultures on malt agar varied in texture from soft to rather tough and in degree of glossiness and pseudomycelium formation. One culture had an atypical assimilation pattern, but it was felt undesirable to describe it as a different species at this time. *C. silvicola* was found in species of *Ips* and in species of *Dendroctonus* in all pines studied. Since these yeasts are nitrate positive, they were compared to species of *Hansenula* (Wickerham, **1951**). They show some resemblance to *H. silvicola* although there are certain differences, primarily in the assimilation of certain of the additional carbon compounds introduced by Wickerham, and by the failure to produce ascospores. If judged only by the criteria used by Lodder and van Rij (**1952**), the organisms might be considered as imperfect forms of *H. silvicola.* In view of the discoveries of several haploid mating types in species of *Candida* by Wickerham and coworkers (**1951, 1954**), groups of our isolates were mixed on various sporulation media. This technique did not induce our strains to sporu-

late. Later, a number of representative strains were sent to Wickerham, who informed us that he had in his collection complementary mating types for our strains. Wickerham believes that the strains differ from *H. silvicola* and he intends to describe the perfect form as a new species of *Hansenula* in a forthcoming publication. Since other investigators are likely to isolate strains of this yeast in its imperfect form, we are giving the description of *Candida silvicola*.

Candida silvicola sp. nov.

In musto maltato cellulae ovoideae, 1.8–4.0 × 2.0–7.0 μ, singulae ad binae. Pseudomycelium abundat. Pellicula non formantur super media liquida. Glucosum fermentatur. Glucosum, galactosum, maltosum, sucrosum, trehalosum, rhamnosum, erythritolum et alpha-methylglucosidum assimilatur, at non lactosum et raffinosum. In medio cum alcohole aethylico crescit. Arbutinum parum finditur.

Growth in malt extract: after 24 hours at 25° C cells round to oval, 1.8–4.0 × 2.0–7.0 μ, single or in pairs. In old cultures a complete white film may be formed. Streak culture on malt agar: after 3 weeks, cream white; some strains semi-dull, others glossy. Texture varies from rather tough to pasty or nearly slimy; smooth, low convex, border shows pseudomycelium. Slide culture on potato agar dextrose: pseudomycelium well developed, some true mycelium, blastospores and blastoconidia. Fermentation: glucose +, galactose − (or fermented very weakly). The other sugars are not fermented. The following compounds are assimilated: glucose, galactose, sucrose, maltose, sorbose, cellobiose, trehalose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, glycerol, adonitol, mannitol, sorbitol, alpha-methylglucoside, salicin, gluconic acid and succinic acid. The following compounds are often used latently or weakly: ethanol, i-erythritol, dulcitol, Ca-2-ketogluconate and citrate. Potassium nitrate is utilized. Starchlike compounds are not synthesized. Added vitamins are required in synthetic media. Splitting of arbutin: positive.

Culture *52–150* isolated from *Ips emarginatus* in *Pinus jeffreyi* has been designated as the type.

The isolates of *C. curvata* agreed fairly well with the description of Lodder and van Rij (**1952**) except that the assimilation of lactose was weak or latent in all cases rather than positive and the strains fermented glucose weakly instead of showing a negative fermentation reaction. The pellicle characteristics varied from an incomplete to a complete film on liquid wort. The two cultures termed *C. parapsilosis* were quite similar to the published description, except that the fermentation of glucose was rather weak.

The isolates of *C. mycoderma* and *C. rugosa* agreed well with the descriptions by Lodder and van Rij except that the last species has a reduced pseudomycelium.

E. TORULOPSIS: Three isolates, of which two were identical, belong to this genus. Both species were distinctly different from any of the described members of this genus. The single isolate could not be identified with any described species of *Torulopsis,* since it is non-fermentative or ferments glucose only extremely weakly and it assimilates glucose, galactose and nitrate. Because of its good growth with nitrate as the single nitrogen source the name *Torulopsis nitratophila* is proposed. It was isolated from *D. monticolae* occurring in *Pinus ponderosa.*

Torulopsis nitratophila sp. nov.

In musto maltato cellulae rotundae ad ovoideae, 1.8–4.4 × 2.1–5.2 μ, singulae, binae aut catenatae. Pseudomycelium nullum. Pellicula non formatur super media liquida. Fermentatur glucosum exiguum. Nitras kalicus assimilatur. Glucosum, galactosum, trehalosum et adonitolum assimilatur, at non sucrosum, maltosum et lactosum. In medio cum alcohole aethylico non crescit. Arbutinum non finditur.

Growth in malt extract: after 3 days at 25° C cells oval to spherical, single, in pairs and in small clusters, 1.8–4.4 × 2.1–5.2 μ. No pellicle, but a thin ring and a moderate sediment are formed in older cultures. Streak culture on malt agar: after 3 weeks greyish to cream colored, smooth, glistening, pasty, low convex with an entire border. Slide cultures on potato agar dextrose: no pseudomycelium formation. Fermentation: only glucose is fermented (latently). The following carbon compounds are assimilated: D-glucose, D-galactose, L-sorbose, trehalose, xylose, L-arabinose, D-arabinose (weak), D-ribose (weak), rhamnose (latent), ethanol (latent), glycerol, adonitol, mannitol, sorbitol. The other compounds are not utilized. Starchlike compounds not synthesized, added vitamins are required in liquid media. Assimilation of nitrate: positive. Splitting of arbutin: negative. Acid production on chalk agar: negative.

The other two isolates belonging to *Torulopsis* were morphologically rather similar to *T. pinus* Lodder and van Rij, but after our isolates were compared with the type species of *T. pinus* numerous differences in carbon assimilation reactions were observed. The cultures have the rather unusual property of utilizing inositol as the single source of carbon. In addition it has the remarkable ability of utilizing melibiose well, but not the corresponding trisaccharide raffinose or sucrose. Because of the last mentioned property the name *Torulopsis melibiosum* is

proposed for these isolates. One isolate was obtained from an adult *D. monticolae* in *Pinus ponderosa* from El Dorado County. The second isolate came from a pupa of *D. monticolae* in *Pinus ponderosa* from Lassen County. The last isolate, No. *52–87*, has been chosen as the type species.

Torulopsis melibiosum sp. nov.

In musto maltato cellulae rotundae aut subovoideae, 2.0–5.3 × 2.5–5.3 μ, singulae aut binae. Sedimentum et anulus subtilis formantur. In agaro maltato cultura mucosa, nitidissima, glabra, margine glabro. Pseudomycelium nullum. Amylum non formatur. Fermentatio nulla. In medio minerali cum glucoso, galactoso, sorboso, cellobioso, lactoso, melibioso, xyloso, D-arabinoso, L-arabinoso, alcohole aethylico, glycerolum, adonitolum, sorbitolum, inositolum bene crescit. Nitras kalicus non assimilatur. Necessariae ad crescentiam sunt vitamina externae.

Growth in malt extract: after 3 days at 25° C cells round to short oval, 2.0–5.3 × 2.5–5.3 μ, single or in pairs. A pellicle is not formed, but in older cultures a delicate ring and a moderate sediment are formed. Streak culture on malt agar: cream colored, smooth, moist glistening, rather slimy, flat with an entire border. Slide cultures on potato agar dextrose: no pseudomycelium. Fermentation: absent. The following carbon compounds are assimilated: glucose, galactose, sorbose, cellobiose, lactose (latent), melibiose, xylose, L-arabinose, D-arabinose (one strain latent), D-ribose (weak), ethanol, glycerol, adonitol (one strain latent), mannitol (weak), sorbitol, salicin (latent), Ca-2-ketogluconate, K-5-ketogluconate, and i-inositol. Starchlike compounds are not synthesized. An external source of vitamins required in synthetic media. Assimilation of nitrate: absent. Splitting of arbutin: negative. Acid production of chalk agar: absent.

It should be mentioned that if the salts of the organic acids are used in the liquid synthetic medium a weak growth was obtained with lactate and citrate and good growth with gluconate and succinate.

E. RHODOTORULA: One isolate was obtained from a *D. jeffreyi* adult taken from *Pinus jeffreyi* in Lassen County. On wort agar it produced a dark yellow color, extractable in petroleum ether by the method of Nakayama *et al.* (**1954**). Since this culture did not produce starch-like compounds under suitable experimental conditions, it belongs in the genus *Rhodotorula*. Its physiological properties are distinctly different from other yellow members of this genus. For this reason it is described as a new species. The name *Rhodotorula crocea* is proposed because of its typical dark yellow color.

Rhodotorula crocea sp. nov.

In musto maltato cellulae ovoideae aut elongatae, 1.8–3.5 × 3.5–7.0 μ, singulae ad binae. Pseudomycelium nullum. Pellicula non formatur super media liquida. Nulla fermentatio. Amylosum non formatum. Glucosum, galactosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum et acidum gluconicum assimilatur, non nitras kalicus et lactosum. In medio cum alcohole aethylico crescit. Arbutinum non finditur. Cultura (post unum mensem, circa 25° C.) crocea, glabra, non mucosa.

Growth in malt extract: after 3 days at 25° C cells are oval to long oval, 1.8–3.5 × 3.5–7.0 μ, single or in pairs. In many cells the ratio of length to width is 3 or 4 to 1. A ring and sediment develop gradually and occasionally a thin pellicle forms. Slide cultures: on potato agar

TABLE II

MAJOR YEASTS ASSOCIATED WITH VARIOUS SPECIES OF IPS AND DENDROCTONUS DURING DIFFERENT STAGES IN THE LIFE CYCLE. A = ADULTS BEFORE EMERGENCE; AF = ADULTS CAUGHT IN FLIGHT; L = LARVAE; P = PUPAE; CA = CALLOW ADULTS

	S. pini					*H. capsulata*					*C. silvicola*					*C. curvata*					Miscellaneous yeasts
	A	AF	L	P	CA	A	AF	L	P	CA	A	AF	L	P	CA	A	AF	L	P	CA	
D. brevicomis (22+; 11−)[1]	7	4	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
D. monticolae (62+; 10−)	4	1	2	0	2	15	0	0	6	6	6	2	6	5	3	9	0	1	0	0	5
D. valens (2+)	0	*	*	*	*	0	*	*	*	*	2	*	*	*	*	0	*	*	*	*	0
D. jeffreyi (8+; 6−)	0	*	*	*	*	6	*	*	*	*	4	*	*	*	*	0	*	*	*	*	4
I. confusus (12+; 6−)	4	*	*	*	*	1	*	*	*	*	3	*	*	*	*	4	*	*	*	*	2
I. oregoni (33+; 7−)	1	0	0	0	0	1	0	0	1	0	17	0	7	4	5	1	0	0	0	0	1
I. emarginatus (6+; 3−)	0	*	*	*	*	0	*	*	*	*	6	*	*	*	*	0	*	*	*	*	1

[1] Figures in parentheses indicate number of individuals yielding yeasts (+), yielding no yeasts (−).
* Isolations not attempted.

dextrose no pseudomycelium is formed. Streak culture: on wort agar after 3 weeks the color is dark yellow, surface smooth with transverse sectors, semi-glistening, texture pasty but not slimy, form convex with entire border. Fermentation: absent. The following carbon compounds are assimilated: glucose, galactose, sucrose, maltose, glycerol, gluconic acid, Ca-2-ketogluconate, K-5-ketogluconate. Cellobiose, trehalose, melezitose, D-mannitol and citric acid are used latently. Other carbon compounds tested, including ethylalcohol, are not used. Assimi-

lation of potassium nitrate: absent. Splitting of arbutin: absent or slightly positive.

Three isolates were obtained belonging to the genus *Cryptococcus*, since they produce starch-like compounds under suitable conditions and do not ferment. The two isolates of *Cr. diffluens* and the one of *Cr. neoformans* (all from *Dendroctonus* beetles in *P. ponderosa*) checked well with the descriptions given by Lodder and van Rij. The last named organism is usually isolated from human cases of cryptococcosis, although Emmons (**1951**) has reported isolations from soil.

Ecology of the yeasts: In Tables II and III the relations are presented between the four principal species found and the beetles and trees from which they were isolated.

TABLE III

YEASTS ISOLATED FROM SPECIES OF DENDROCTONUS AND IPS ATTACKING VARIOUS PINE TREES

	Species of yeast									
	H. capsulata		*C. silvicola*		*C. curvata*		*Sacch. pini*		Misc. group	
Species of tree	*D.*[1]	*Ips*[2]	*D.*	*Ips*	*D.*	*Ips*	*D.*	*Ips*	*D.*	*Ips*
Pinus jeffreyi	6	1	5	24	0	0	0	0	4	1
Pinus ponderosa	17	2	9	5	9	4	21	2	7	2
Pinus monticola	7	0	10	1	0	0	6	2	1	1
Pinus contorta var. *latifolia*	3	0	4	11	1	1	0	1	0	0
Pinus lambertiana	0	0	0	1	0	0	0	0	0	0

[1,2] = Yeasts isolated from species of *Dendroctonus* and *Ips*, respectively.

Because of the limited scope of the survey, it is not possible to draw definite conclusions as to the association of certain species of yeasts with certain bark beetles and the trees in which they occur. There is little doubt, however, that *H. capsulata* and *C. silvicola* are of the most general occurrence, since they were isolated from beetles in all pines studied. The fact that *Pinus lambertiana* is an exception is not serious, since only a single sample was taken from this species of tree. *S. pini* was also of general occurrence, except that it was never isolated from beetles collected in *Pinus jeffreyi*. Whether this is due to the limited scope of the survey or due to the presence of certain compounds in the oleoresins (such as n-heptane, which is the major constituent of the turpentine of *P. jeffreyi*) to which *S. pini* might be sensitive, cannot be decided at the present time. It would also be worthwhile to confirm the

observations (see TABLE II) that *S. pini* appears to be the most common yeast in *D. brevicomis* and *C. silvicola* was most prevalent in *I. oregoni*. In *D. monticolae*, on the other hand, all major species of yeasts were found.

It is also interesting that yeasts were isolated regularly from the non-feeding metamorphic stages, such as pupae and callow adults (see TABLES II and IV). Since the exterior of the brood material was carefully sterilized, it must be concluded that the yeasts are more intimately associated with the beetles than merely forming a part of their nutrition during the feeding stages.

TABLE IV

NUMBER OF BEETLES DISSECTED IN THE DIFFERENT STAGES OF THEIR LIFE CYCLE AND THE CORRESPONDING NUMBER OF YEASTS ISOLATED

Stage in life cycle	No. of individuals dissected	No. of individuals yielding no yeasts	No. of isolates obtained
Adults in flight	9	1	7
Adults before emergence	106	21	102
Callow adults	18	0	19
Pupae	19	2	19
Larvae	26	3	22
Total	178	27	169

DISCUSSION

The findings show that the 169 yeasts isolated from certain species of the barkbeetles *Ips* and *Dendroctonus* occurring in various species of *Pinus* in northern California, belong primarily to 3 well-defined species of yeasts, *viz.*, *Hansenula capsulata* (36 isolates), *Saccharomyces pini* (32 isolates) and *Candida silvicola* (70 isolates). Besides these 3 main species, several miscellaneous yeasts were present in smaller numbers. Of the miscellaneous group 4 yeasts were described as new species, since they could not be identified with any of the known species. The proposed names are *Pichia haplophila, Torulopsis nitratophila, Torulopsis melibiosum,* and *Rhodotorula crocea.*

For the classification of the yeasts we have used in general the system of Lodder and van Rij (**1952**) and for *Hansenula* that of Wickerham (**1951**). The disadvantage of the first system is that several yeasts with distinctly different biochemical properties often are grouped together in one species, because of the rather limited number of physiological reactions used. An example of this is the grouping together of *S. pastori* and *S. pini* by the Dutch workers. Since *S. pini* appeared

to be one of the important bark beetle yeasts we compared the properties of our isolates with data pertaining to type species of *S. pini* and *S. pastori* which were kindly made available to us by Dr. Wickerham. All the isolates concerned were identical with *S. pini*, whereas *S. pastori* was not found (*cf.* review of the literature). Of the other isolates collected, only those were described as new species which could not be classified using the system of Lodder and van Rij or by descriptions in other publications.

Among the noteworthy physiological characters of our isolates are the abilities of all principal species and most of the miscellaneous yeasts to assimilate cellobiose and xylose, and to a somewhat lesser extent certain of the other pentose sugars. These abilities may be related to the fact that the beetles feed on the phloem of the tree, presumably followed by digestion of the hexosans and pentosans present. It might be speculated then that some of the hydrolysis products of the wood polysaccharides are also substrates for the yeasts associated with the beetles. Another interesting feature of the yeast isolates is their poor fermentative power. No sugars, besides glucose, are fermented by any of the isolates and in most cases, even the fermentation of glucose is weak or negative. Apparently, the environmental conditions under which these yeasts live are more favorable for an oxidative type of metabolism. Furthermore, the percentage of yeasts (64%) able to utilize nitrate as the single source of nitrogen is unusually high as compared to most published surveys of yeasts. Whether nitrate is one of the principal sources of nitrogen available in the phloem sap or whether there are other reasons for this phenomenon is difficult to say, since there does not appear to be sound data in the literature on this point (*cf.* Burström, **1946**).

SUMMARY

A survey was made of the yeasts occurring in different metamorphic stages of the bark beetles *Dendroctonus brevicomis, D. monticolae, D. jeffreyi, D. valens, Ips oregoni, I. confusus,* and *I. emarginatus.* The exterior parts of the insects were sterilized and all isolations were made, after dissection, from the interior part. The host trees studied were *Pinus ponderosa, P. jeffreyi, P. monticola, P. contorta* var. *latifolia* and *P. lambertiana,* all growing in Northern California. One hundred and sixty-nine yeasts were isolated and identified. The taxonomic designation and numbers of isolates obtained were as follows: *Saccharomyces pini* (32), *Hansenula capsulata* (36), *Pichia haplophila* (3), *Candida silvicola* (70), *C. curvata* (15), *C. parapsilosis* (2), *C. mycoderma* (3),

C. rugosa (1), *Torulopsis nitratophila* (1), *T. melibiosum* (2), *Rhodotorula crocea* (1), *Cryptococcus diffluens* (2), *Cr. neoformans* (1). The results tend to show that *S. pini* is the most common yeast found in *D. brevicomis* and *C. silvicola* is most prevalent in *I. oregoni*, while *D. monticolae* contained representatives of all three major species of yeast. Certain typical physiological properties of the yeast isolates are discussed.

ACKNOWLEDGMENT

The authors are greatly indebted to Dr. L. J. Wickerham for the many valuable suggestions made in connection with the identification of the yeasts. We also wish to extend our thanks to Dr. R. Z. Callaham for supplying some of the bark beetles and their identification and to the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, Forest Insect Investigations in Berkeley, for supporting part of the investigations.

DEPARTMENT OF FOOD TECHNOLOGY,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

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THE PERFECT STAGE OF HORMODENDRUM PEDROSOI

CÉSAR A. FUENTES AND FREDERICK A. WOLF

(WITH 2 FIGURES)

Hormodendrum pedrosoi Brumpt is a polymorphic, imperfect fungus of the family Dematiaceae, and is one of the agents of chromoblastomycosis, a chronic dermatitis of man that is endemic in tropical and subtropical regions. Cases have been reported, however, in temperate regions, as in New Zealand, South Africa, Russia, and the United States. For the reason that this fungus varies so greatly in the type of reproductive structures produced, it has been given at least 7 different generic names and 6 different specific names. The confusing problem of its proper nomenclature and classification, therefore, has long awaited the discovery of a perfect or ascosporic stage. Recently Ajello and Runyon (**1953**) found abortive perithecia in the closely related *Phialophora verrucosa* Thaxter. Also Karrer and Conant (**1953**) found cleistothecium-like bodies in an isolate of *H. pedrosoi* that had been maintained in culture for several years in the Laboratories of Medical Mycology at Duke University. However, asci and ascospores never developed within these bodies even though they mated numerous strains. Nevertheless their observations stimulated us to search for ascocarps in cultures isolated from cases of chromoblastomycosis in Cuba, and also in cultures sent to us from other areas. As a result, certain strains were found to produce fertile ascocarps, and the present purpose is to report these findings and their interpretation.

MATERIAL AND METHODS

The material studied consisted of 16 isolates of *H. pedrosoi* obtained from cases of chromoblastomycosis in Cuba, 15 of which were made available by Dr. José Alfonso Armenteros and the other one by Dr. Fernando Trespalacios. The tissues of lesions in all cases contained dark, nodular, sclerotic bodies, characteristic of the pathological findings in this disease. For comparative study, an isolate of *H. compactum* Carrión, supplied by Dr. N. F. Conant, Duke University, and one of

Phialophora verrucosa, sent by Dr. L. Ajello, Public Health Service, Atlanta, Ga., were examined.

The cultures of *H. pedrosoi,* at the time of our first examination, had been maintained without transfer for periods varying from 5 to 36 months. All had been grown on Sabouraud's dextrose agar, 11 in tubes with cotton stoppers and 5 in tubes with cork stoppers. All were severely desiccated, those in the cork-stoppered tubes being somewhat less desiccated. All were transferred to tubes of fresh Sabouraud's agar containing 40 g dextrose, 10 g peptone to 1 liter of water, with added antibiotics (500 micrograms of Actidione, 80 units of Penicillin, and 160 units of Streptomycin to suppress contaminating fungi present in some of the cultures). All cultures were maintained at room temperature. The strains called Jacomino and No. *1885* were also planted on maize meal agar. On these media 4 isolates, Nos. *631, 1885, 832,* and Jacomino, produced both ascocarps and conidia. All cultures had so-called sclerotic cells, a feature unknown among non-pathogenic species of *Hormodendrum.* Two strains, Nos. *969* and *1035,* formed sclerotial bodies and conidia. All strains bore conidia characteristic of *H. pedrosoi.*

From time to time after black bodies, the ascocarps, began to appear in the cultures of Nos. *631, 1885, 832,* and Jacomino, direct microscopic examinations were made by removing these bodies, and crushing them to disclose their content by pressing upon the cover glass. Also, microcultures were prepared and examined repeatedly to follow the course of ascocarpic and conidial development. As another means of following their development, materials were appropriately fixed, embedded in paraffin, sectioned, and stained.

RESULTS

Brief consideration will be given, in turn, to each of the 4 ascocarp-producing isolates, as follows:

No. *631*—This isolate was a 35-month-old culture in a cork-stoppered tube in which the medium had shrunken in volume by approximately one-half. It contained numerous, spherical, black bodies, varying greatly in size. The smaller ones, 45 to 60 μ in diameter, when crushed, appeared to be sclerotial throughout. The larger ones were ostiolate (FIG. 2, D), measuring 120–200 μ in diameter, and, on fracture, were found to be empty or else yielded a spore-laden, yellowish, mucilaginous content (FIG. 2, B). These spores were presumed to be ascospores even though no asci could be found. They were reniform when viewed laterally and elongate-elliptical when seen dorsally or ventrally. They

were dilute brown and measured 8.4–10.5 × 3.2–3.8 μ (FIGS. 1, D, 2, F). The crushed empty fruit bodies revealed that the wall was constituted of several layers of thick-walled, dark, polygonal cells (FIG. 2, C).

When this isolate was transferred to fresh media it grew slowly and only immature ascocarps were found after 4 months.

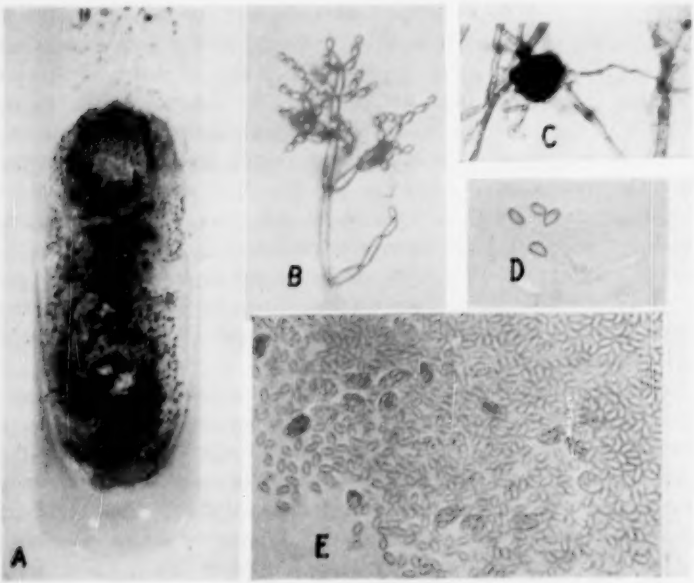

FIG. 1. A. *Microascus pedrosoi* in culture on Sabouraud's agar. B. Conidiophore and conidia of the *Hormodendrum pedrosoi* stage. C. Sclerotial body from which conidiophores are borne. D. Ascospores, one of which has germinated. E. Mucoid material in which ascospores and asci are embedded.

No. *1885*—This strain was represented by 3 cultures in cork-stoppered tubes, one of them 2 months old and the other two 34 months old. Each contained ascocarps like those in No. *631*. When some of them were broken open, it was noted that they were filled with a mucilaginous spore-laden material which contained also numerous, intact groups of 8 spores without a defined enveloping membrane. Six plantings were made on maize meal agar and 3 on Sabouraud's agar to which sufficient lactic acid was added to lower the pH to 4.3. Mycelial growth proceeded slowly and remained sparse but after 4 weeks ascocarps began to form on these acidulated media. Eventually they

were found to contain groups of spores embedded in a mucilaginous matrix. These groups were regarded as unwalled asci, and measured 13–14 × 6.5–7 μ. Microcultures of such spores on Sabouraud's agar yielded conidiophores and conidia typical of *Hormodendrum pedrosoi*

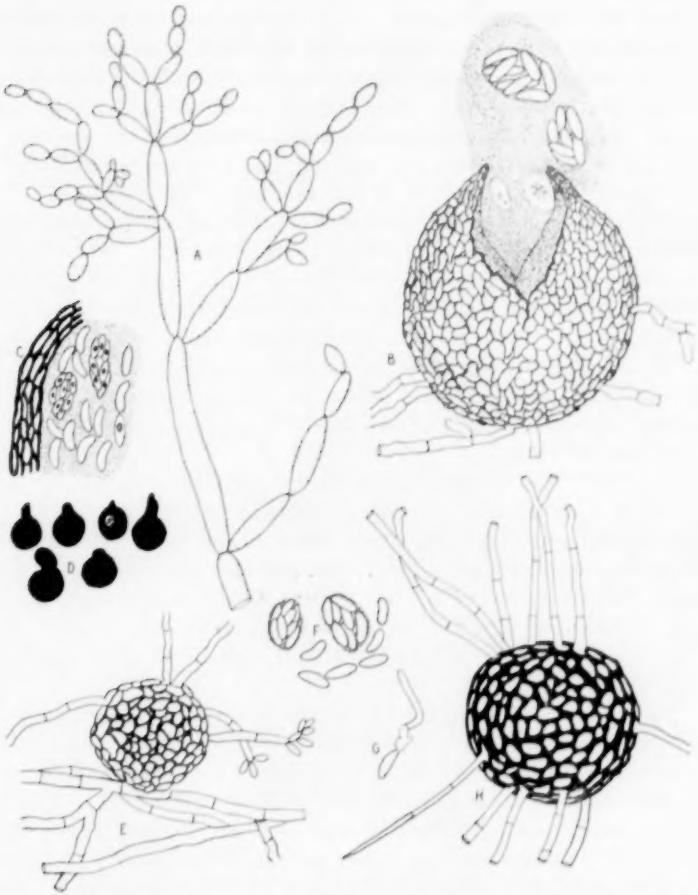

FIG. 2. Sketches of structures of *M. pedrosoi*, made from photographs. A. *Hormodendrum pedrosoi*. B. Crushed haerothecium of *M. pedrosoi*. C. Portion of wall of the ascocarp, and asci, and ascospores embedded in mucoid matrix. *D*. Silhouettes of ascocarps of *M. pedrosoi*. E. Sclerotial body bearing conidiophores. F. Asci and ascospores. G. Germinating ascospore. H. Sclerotium, presumably an immature haerothecium.

(FIGS. 1, B; 2, A). And when some of these ascospores were planted in tube cultures they again gave rise to ascocarps in abundance.

Blocks of agar containing ascocarps were removed from 3-month-old cultures, were fixed in formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. These sections, on examination, showed the following features. (1) The ascocarps are globular but provided with a papilla or beak. (2) The wall is composed of 3 to 4 layers of dark, thick-walled cells (FIG. 2, C). (3) A pore extends through the ascocarp apex, providing an ostiolum. (4) Groups of 8 ascospores, lacking a definitive enclosing membrane, are disposed at different levels throughout the ascocarp, i.e., they are not arranged in a hymenial layer at the floor of the ascocarp (FIG. 2, C). (5) The medullary portion of young ascocarps in which ascospores had not yet formed, consists of thin-walled fungus tissue, presumably "nurse" tissue. (6) Paraphyses and periphyses are lacking. (7) Groups of ascospores in 8's occur near the walls but are not found near the center of unopened ascocarps. (8) As the nurse tissue disappears, beginning in the central part of the locule, it is replaced by mucoid material which together with the ascospores fills the locule before an opening is effected. (9) On opening, mucoid material and embedded ascospores accumulate in masses at the ostiolar orifice.

No. *832*—This isolate was a 4-month-old culture, completely dehydrated, in a cotton-stoppered tube. Many ascocarps were present that were of the same shape and size as those in the other fertile cultures. When they were crushed no asci were seen but ascospores were abundant. Three transfers of this isolate were made to Sabouraud's dextrose agar, in only one of which ascocarps began to develop after 15 days. Even though the other two cultures were maintained for 7 months no fruit bodies were found.

Jacomino—This 24-month-old isolate in a cork-stoppered tube was in good condition and bore many beaked ascocarps approximately 275 μ in diameter (FIG. 1, A). In addition, there were spherical bodies with septate hyphae extending from their surface (FIG. 2, H). Conidiophores arose from the surface of some of these spherical bodies (FIGS. 1, C; 2, E). When the fruit bodies were fractured under a cover glass, ovoid 8-spored asci lacking a definite ascal membrane were freed. These asci and ascospores were similar in size and shape to those of the other isolates (FIG. 1, E).

Three transfers of this strain to Sabouraud's dextrose agar were made, and after 7 weeks the colonies of each were velvety and dark gray, and mature carbonaceous ascocarps were abundantly present. Sections

prepared from one culture revealed empty locules, locules containing characteristic ascospores in groups but without a discernible ascus wall, and others that were immature, having locules filled with amorphous material. Ascospores from one of these transfers, used as inoculum for a slide microculture, developed mycelium bearing *H. pedrosoi* conidiophores and conidia.

The failure of 12 of the 16 strains employed in this study to form ascocarps remains wholly without explanation.

INTERPRETATION

Manifestly the salient features of this ascocarpic fungus accord with those of the genus *Microascus,* first described by Zukal (**1885, 1890**), as a perithecium-forming organism among the Sphaeriales of the family, Ceratostomellaceae. However, Fischer (**1897**), disregarding the presence of an ostiolum and emphasizing instead the irregular arrangement of the asci within the ascocarps, transferred *Microascus* to the cleistothecium-forming fungi, and placed it in the Eurotiales of the family Aspergillaceae. This change, until recently, appears to have met with general approval by all who have dealt with this genus. In 1947 Falck and Falck (**1947**), however, proposed that a new class Haerangiomycetes be employed for fungi possessing the following characteristics: (1) ostiolate ascocarps; (2) asci lacking a definitive ascal wall; (3) asci borne at different levels within the ascocarp; (4) ascospores in groups surrounded by a mucoid matrix, all of which exude from the ostiolum en masse to form a droplet or cirrhus; (5) ascospores borne in an octophore, i.e., arranged in an octet around an axis, somewhat as the segments of an orange are arranged. Such an ascocarp therefore is neither a perithecium, nor a cleistothecium, but must be termed an haerothecium (haero = to adhere or stick, thecium = spore case). The structure of the fungus under consideration is believed to be of this kind, hence it is being placed among the Haerangiomycetes.

A search of the literature reveals that 8 species have been definitely assigned to the genus *Microascus.* For comparison, their essential features are given in TABLE I.

A conidial stage, identified as *Scopulariopsis,* occurs in connection with *M. trigonosporus* and *M. lunasporus.* Whether such identification is correct is questionable since *Scopulariopsis* is moniliaceous and the ascosporic stages of these 2 species are dark-brown or carbonous. It would therefore be anticipated that their conidial stages also would be pigmented, and therefore would be dematiaceous, belonging in *Hormo-*

TABLE I
SPECIES OF MICROASCUS

Organism	Habitat	Haerothecium		Ascus		Ascospores	
		Size	Shape	Size	Shape	Size	Shape
M. longirostris Zukal	dog dung	400μ tall	beaked	30–36μ	globose	4–5×2–3μ	acute lanceolate
M. sordidus Zukal	olive leaves	200–300μ diam.	pyriform	13–15×12–13μ	pyriform	9–9.5×5–6μ	reniform
M. variabilis Massee & Salm.	dung	150–200μ diam.	papilliform	7–8μ	subglobose	3–3.5μ	lunate
M. nidicola Massee & Salm.	old nest of *Bombus*	330μ diam.	short conic	10–13×6–8μ	globose-elliptic	7.5–8×2μ	sublunate
M. trigonosporus Emmons & Dodge	cereal and legume seed and dermal lesion	125–235μ diam.	beaked	8–11×7–9μ	oval	4.5–5×3–3.8μ	triangular
M. intermedius Emmons & Dodge	strawberry roots	75–150μ diam.	pyriform	10–11×7.5–10μ	oval to elliptic	3.5–5.5μ	conic-convex
M. lunasporus Jones	dermal lesion	175–300μ diam.	beaked	9–14×7–12μ	oval	8–14×4–7μ	lunate
M. cirrosus Curzi	*Prunus* leaves	90–160μ diam.	long beaked	10–15×7–10μ	globose-elliptic	8–14×4–7μ	calciform

dendrum. Curzi (**1931**), in fact, regarded the conidial stage of *M. cirrosus* as dematiaceous but did not place it in any form genus.

It has been brought into question whether certain other described fungi should be included in *Microascus*. Among these are *Peristomium desmosporium* Lechmere (**1913**), *Scopulariopsis cinerea* Emile-Weil & Gaudin (**1919**), and *Dematophora glomerata* Viala (Curzi, **1935**). The structure of the ascocarps of these appears to be generically alike, and their development appears to resemble that of *M. trigonosporus*, as described by Emmons and Dodge (**1931**).

The ascocarpic stage of *Hormodendrum pedrosoi* is most nearly of the same shape and dimensions as *M. lunasporus*. However, cultures of *M. lunasporus* are no longer available for comparison. It is believed, however, on the basis of the description and illustrations given by Jones (**1919**), that *M. lunasporus* is distinct from the fungus under consideration. The ascocarpic stage of *H. pedrosoi* is therefore given the new name, *Microascus pedrosoi*. The following brief Latin description summarizes the salient features of this new species.

Microascus pedrosoi sp. nov.

Haerotheciis sparsis, nigris vel carbonaceis, globosis, 120–200 μ diam.; rostellis crassis, papilliformibus vel circiter aequalibus diam. partis globosae haerothecii; contexto rigido ex cellulis nigro-fuscis composito. Ascis gelatinoso-tunicatis, globoso-ellipticis, numerosis, octosporis, 13–14 $\times$ 6.5–7 μ, quae ante plenam ascosporidiorum maturitatem solventur et muco mixta ascosporidia in massa orieuntur quae ex ostiolo expellitur. Sporidiis ferrugineis vel dilute brunneis, continuis, lunulatis, utrinque obtusis, levibus, 8.4–10.5 $\times$ 3.2–3.8 μ.

Status conidicus *Hormodendrum pedrosoi* est. Hyphis fructiferis dematiaceis, erectis, irregulariter ramosis. Ramulis ultimis catenulas conidiorum gerentibus. Conidiis ovoideis, continuis, brunneis, 3–6 $\times$ 3 μ.

Hab. in hominibus in quibus dermatites generat.

SUMMARY

This account deals with the ascocarpic stage of *Hormodendrum pedrosoi*. Four among 16 strains isolated from cases of chromoblastomycosis developed ascocarps on Sabouraud's agar or maize meal agar. The ascogenous stage is identified and described as a new species, *Microascus pedrosoi*. It belongs among the Haerangiomycetes.

UNIVERSIDAD DE HABANA,
ESCUELA DE MEDICINA,
HABANA, CUBA
AND
DUKE UNIVERSITY,
DEPARTMENT OF BOTANY,
DURHAM, N. C.

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STUDIES ON TRICHOPHYTON TONSURANS. I. THE TAXONOMY OF T. TONSURANS

LUCILLE K. GEORG [1]

(WITH 2 FIGURES)

Ringworm infections due to *Trichophyton tonsurans* are becoming an increasingly important public health problem in the United States (**15**). Since the clinical aspects of the disease have been discussed in recent publications (**22**, **23**, **35**, **36**), they will not be reviewed here. In general, the fungus invades the skin, hair, and nails of both children and adults and produces chronic, superficial, or acute suppurative infections. These infections may simulate other dermatological conditions such as seborrhea, eczema, lupus erythematosus, or acute bacterial infections.

Accurate diagnosis is dependent upon direct examination of infected hairs, skin, or nail scrapings for the presence of fungus elements, followed by the isolation and identification of the causative agent. The various factors and problems involved in these laboratory procedures are presented in this report, along with a discussion of the classification and synonymy of the fungus, *T. tonsurans*.

HISTORY

T. tonsurans was probably first seen in clinical materials by Gruby in 1844 (**20**). Gruby described but did not name a fungus in hairs and skin scrapings from cases he spoke of as being the "teigne tondante de Mahon" or "herpes tonsurans de Cazenave."

The following year, 1845, Malmstem (**26**) verified the studies of Gruby by publishing in detail a description of a similar clinical entity complete with descriptions and illustrations of the fungus in clinical materials. His drawings clearly show that the fungus elements were confined to the interior of the hairs, thus producing a characteristic endothrix picture. He proposed the name *Trichophyton tonsurans* for the etiologic agent. However, although Malmstem clearly proposed the use of the generic name *Trichophyton*, which he had derived from the Greek words meaning hair and plant, he also suggested that "one might

[1] From the Communicable Disease Center, Public Health Service, United States Department of Health, Education and Welfare, Chamblee, Ga.

rather call it *Trichomyces* from the Greek words meaning hair and fungus." He stated, however, that he preferred the name *Trichophyton*. Since the second name was only suggested and not recommended, it should be discarded. Although Malmstem did not isolate and grow the fungus in culture, validity of the species and the name *Trichophyton tonsurans* has been assigned to him by most of the later authors who have studied the fungus from the disease he described.

Unfortunately, loose usage by early workers of the name *T. tonsurans* for fungi isolated from various clinical entities led to much confusion and error in the taxonomy of this fungus and its many morphological variants. Mégnin, for example, in his early studies of disease in animals, in 1878 (**29**) pointed out that there were two distinct species of *Trichophyton* which were responsible for ringworm in animals. The common ringworm of the horse, which he stated had the same characteristics as that seen in dogs and cats, he ascribed to *T. tonsurans*. For the fungus agent responsible for ringworm in cattle, he gave the name *T. depilans*. Mégnin's use of the name *T. tonsurans* for an agent of ringworm in horses was indeed unfortunate; however, his descriptions of the disease, as well as his drawings of the infected hairs showing chains of spores 2 to 3 μ in diameter on the outsides of the hairs, make it very clear that he was dealing with an ectothrix *Trichophyton*, probably *T. metagrophytes*, which we now know to be a common cause of ringworm in the horse. The *T. depilans* which he described from cattle ringworm appears also to be an ectothrix *Trichophyton*. Here the spores on the outside of the hairs averaged 5 to 6 μ in diameter. This fungus was probably the same organism which Bodin later described clearly and named *T. verrucosum*, the fungus we now know as the common cause of ringworm in cattle.

Nine years later, in 1887, Mégnin, in collaboration with Boucher (**5**), again described ringworm in cattle. Here, however, he used the name *T. epilans* for the causative agent; but his description of the disease in the calf, as well as the manner of the hair invasion, is identical with that ascribed to *T. depilans* in the earlier paper. Both names, *T. epilans* and *T. depilans*, must be considered as nomina ambigua, as cultures were not described, and even the author himself seems to have been uncertain as to whether he had two different species producing the same clinical picture.

Many later authors were influenced, however, by the early studies of Boucher and Mégnin and somehow acquired the idea that endothrix trichophytons were found associated with ringworm in animals. *T. epilans* indeed was later equated with the *T. cerebriforme* of Sabouraud;

however, a study of the early literature, such as publications by Mégnin and by Boucher and Mégnin, cited above, rules out the validity of this synonymy.

Probably the first to study the fungus of Gruby and Malmsten in culture was Grawitz in 1886 (**17**). This worker gave a clear description of *T. tonsurans* in clinical material, described the fungus colony on culture media, and also apparently reproduced the disease by inoculating his cultures into two persons. Sabouraud (**40**) glossed over this work of Grawitz although he did cite references to his papers.

Zopf in "Die Pilze," in 1890 (**46**), gave Grawitz credit for describing the fungus *T. tonsurans*, and he himself described a number of cases of ringworm of the scalp and beard which are clearly due to this fungus. However, after describing the organism, he placed it in the genus *Oidium* as *O. tonsurans*.

Sabouraud in 1893 (**38**) pointed out that there are two separate groups of large-spored trichophytons: "les mégalotrichophytons animaux" and "les mégalotrichophytons de l'homme." The fungi found in animals he described as having the ability to invade not only the hair itself, but to fill the follicle around the hair with large spores (clearly ectothrix). These species caused three clinical entities. The first, a deep (suppurative) lesion in man of the type commonly acquired from horses from which he isolated a white culture, was undoubtedly *T. mentagrophytes*. The second, which produced a slowly disseminated, moist lesion, the type commonly acquired from cattle, he described as producing a culture which was "jaune, craquelée, vermiculaire." This culture could have been either *T. verrucosum* or *T. mentagrophytes*. The third, which produced a dry type of lesion and which he believed (at that time) was contracted from birds, was probably *T. megninii*.

The fungi found only in humans, "les mégalotrichophytons de l'homme" were described as having the fungus elements confined to the interior of the hairs (clearly endothrix). He divided this group into two categories: one characterized by having a "resistant mycelium in the hair" and a "crateriform" culture, the other as having a "fragile mycelium in the hair" and an "acuminate" culture. These are clearly the two morphologic types which he later described as *T. crateriforme* and *T. acuminatum*.

The following year, 1894, Sabouraud published his first text on the subject, "Les trichophyties humaine" (**39**). Here, particularly in the atlas, where the fungus cultures are described and illustrated, we can be certain that the above assumptions are correct. Here he speaks of the two types of cultures which produce endothrix infections as *T. à*

culture crateriforme and *T. à culture acuminée*. The illustrations and descriptions of the ectothrix fungus from calves which he indicates as "*T. ectothrix à culture jaune, craquelées, vermiculaires*" suggest that the fungus depicted was probably *T. mentagrophytes*. It was described as producing a fairly flat, rapid-growing granular colony with many microconidia which were easily disseminated from the surface, and it showed many "sketchy" (abortive) perithecia. The yellow color and irregularly folded surface is not uncommonly seen in *T. mentagrophytes* strains isolated from animals.

In 1894, Rosenbach (**37**), a German worker, described seven new species of *Trichophyton*. One of these, *T. plicans fusisporum*, has been thought by some authors (**10, 28**) to be a fungus similar to *T. tonsurans* and it has been placed in synonymy with *T. plicatile* of Sabouraud. However, Rosenbach's descriptions of the disease it produced and the fungus itself are very inadequate. The characteristic of producing "many spindle-shaped spores" makes synonymy with *T. tonsurans* very doubtful. The highly folded surface of the culture is certainly not enough evidence for deducing relationship with this organism. The species described by Rosenbach should be considered as a nomen nudum.

Blanchard in 1896 (**3**) reviewed these early studies, and described a form similar to Sabouraud's *T. à culture acuminée*. However, he wished to give the organism a true binomial and named it *T. sabouraudi* in honor of Sabouraud.

In 1902, E. Bodin, a student of Sabouraud, published his book, "Les champignons de l'homme" (**4**). Here he redescribed several species of endothrix fungi, giving binomials where these had been lacking in the early papers of Sabouraud. He proposed the name *T. crateriforme* for the *T. à culture crateriforme* of Sabouraud and, ignoring the *T. sabouraudi* of Blanchard, proposed *T. acuminatum* for Sabouraud's *T. à culture acuminée*. He also, according to Sabouraud (**40**), gave the name *T. flavum* to Sabouraud's *T. jaune craquelée vermiculaire*. This later designation had been given earlier by Sabouraud to cultures which he had isolated from horses and from humans who had had contact with ringworm infected horses. In these cases the infected hairs had been clearly of the ectothrix type. Bodin apparently thought he was dealing with an ectothrix fungus, since he considered the *T. depilans* of Mégnin as a synonym. However, Sabouraud, in 1910, seems to have changed his mind about his early *T. jaune craquelée vermiculaire* and decided that this designation as well as the *T. depilans* and *T. epilans* of Mégnin referred not to an ectothrix fungus but to an endothrix organism. He rejected the name *T. flavum*, proposed by Bodin, as not being

sufficiently descriptive, and gave his own binomial *T. cerebriforme*. This organism he clearly described in "Les teignes" as an endothrix fungus, but he admitted later in the text that both he and Bodin had been mistaken about its having originated from horses. This confusion should certainly place the names *T. flavum* and *T. cerebriforme* in the category of nomina confusa. Furthermore, there is absolutely no basis for equating the *T. epilans* and *T. depilans* of Mégnin with an endothrix species.

Two other endothrix species were assigned to Sabouraud in 1909 by Favera, an Italian worker (**11**): *T. fumatum* and *T. regulaire*.

The most complete description of the endothrix dermatophytes and the disease they produced came with the publication of "Les teignes" by Sabouraud in 1910 (**40**). Here Sabouraud redescribed the five species assigned to him by Bodin and Favera, and added six new species: *T. circonvolutum, T. effractum, T. umbilicatum, T. pilosum,* and *T. plicatile*. He also gave the name *T. sulfureum* to the endothrix fungus with "the primrose crater" described in England by Fox (**13**), and *T. exsiccatum* and *T. polygonum* to the fungi described by Uriburu (**44**) in Argentina. As mentioned above, he changed Bodin's *T. flavum* to *T. cerebriforme* since he thought this later name more descriptive of the fungus; however, this synonymy rules out both names, as the *T. flavum* of Bodin is undoubtedly an ectothrix while the *T. cerebriforme* of Sabouraud is an endothrix fungus.

Sabouraud placed considerable emphasis on the manner of hair invasion as a criterion for species definition. For example, he claimed that *T. cerebriforme* and *T. plicatile* were distinctive in that infected hairs showed not only chains of spores inside the hairs, but that hyphae and similar spore chains developed on the outside of the hair as well. This type of hair invasion was named by Sabouraud neo-endothrix. He also claimed that he could differentiate between infections due to *T. crateriforme* and *T. acuminatum* on a clinical basis. In the crateriform type, the hair stumps were yellowish grey in color and paler than the normal hairs. They tended to break off 2 to 4 millimeters above the scalp. The infected hairs showed long strands of mycelium in their interiors and tended to break into rectangular segments. These fungus structures only partially filled the hairs. What is more, he stated that the mycelium of the crateriform type in hairs and tissue scrapings was more resistant to the action of KOH than was the mycelium of the acuminate type. In infections due to *T. acuminatum*, however, the hair stumps tended to be dark and were broken off close to the scalp, producing the so-called "black dot" picture. These hair stumps were packed with

chains of nearly round spores which easily dispersed when the hair was placed in KOH.

Other authors (**24**, **28**) have carried on these ideas and have made a great point in associating certain clinical pictures with individual species. In the discussion below, it is pointed out, however, that these studies and those of other contemporary workers show that correlation of colony types with manner of hair invasion, resistance of the mycelium to KOH, or with specific clinical signs is entirely fallaceous.

In the years following Sabouraud's studies more than 12 other endothrix species have been described: *T. ochropyrraceum*, *T. fuscum*, *T. bicolor*, *T. cineraceum*, etc. It will be noted that the species names chosen, as the earlier names, are largely descriptive of some striking morphological feature, such as pigmentation of the colony. Indeed, there is little evidence in the various descriptions of any fundamental differences among these "species" to warrant considering them different from one another. The microscopic morphology, although poorly described for many, shows little differences beyond what one might expect to find within the normal range of variation of a fungus species.

Recently, a number of workers have made an attempt to bring logic and order to this problem. Carrión and Silva in 1944 (**6**) made excellent progress in this direction. These authors described and illustrated very clearly that variations in colonial form were common in isolates of *T. tonsurans*. For example, changes from the radial to the cerebriform type folding, or from the cerebriform to the crateriform type and vice versa, would sometimes take place in subcultures of a single isolate, and occasionally in sectors developing in a single colony. They suggested, therefore, that since the type of folding was variable in a single isolate it was an undependable criterion for specific diagnosis. They also pointed out that other differential features among described species, such as degree of branching of the fertile hyphae and the relative abundance of chlamydospores, would seem to fall within the range of species variation. In conclusion, they suggested the possible synonymy of various species described in the literature such as *T. tonsurans*, *T. sabouraudi*, *T. flavum*, and possibly others.

In 1945, González Ochoa and Romo Vázquez (**16**) accepted the synonymy suggested by Carrión and Silva and urged that all of the organisms of the "Crateriform Group" be reduced to one species, *T. tonsurans*.

Among the European workers, this reduction of species has not been so readily accepted. The Spanish workers, Matilla and Peña Yañez in 1950 (**28**), however, have urged a reduction to four species:

T. tonsurans, T. epilans, T. sabouraudi, and *T. sulfureum,* and have listed all other named species as synonyms of these.

However, a more recent publication from Spain by Pereiro Miguens, 1954 (**34**), advises the use of the single name, *T. tonsurans,* to include all four of these "species." He suggests, however, that colonial variation may be designated by the use of variety names, *e.g., T. tonsurans* var. *sulfureum.*

Due to the fact that there has not been complete acceptance of species limits in this group, there still is considerable confusion in the literature with regard to the number of valid species. For this reason it was felt important to review the problem, and to determine as clearly as possible, by morphological, physiological, and clinical studies, the number of species that were founded on sound criteria.

EXPERIMENTAL

Included in this study were 13 strains recently isolated from different specimens of endothrix hairs received by the Communicable Disease Center for diagnostic study. All of these isolates appeared typical of the "Crateriform Group" and had been tentatively identified as *T. tonsurans.* In addition 26 cultures of species within this group obtained from various culture collections were also studied. These strains are listed below:

(1) Communicable Disease Center
13 recent isolates

(2) Sabouraud Collection, Paris (through the kindness of Dr. E. Rivalier, Institute Pasteur, Paris, France)
T. crateriforme (2 isolates)
T. cerebriforme (2 isolates)
T. sulfureum (3 isolates)
T. plicatile (2 isolates)
T. flavum (1 isolate)
T. fumatum (1 isolate)
T. effractum (1 isolate)
T. rotundum (1 isolate)

(3) London School of Hygiene and Tropical Medicine (through the kindness of Dr. R. W. Riddell, Brompton Hospital, London, Great Britain)
T. sulfureum (5 isolates)

(4) Institute Pasteur of Algeria (through the kindness of Dr. A. Catanei, Institute Pasteur, Algiers, Algeria)
T. acuminatum (1 isolate)
T. fumatum (1 isolate)
T. umbilicatum (1 isolate)
T. flavum (1 isolate)

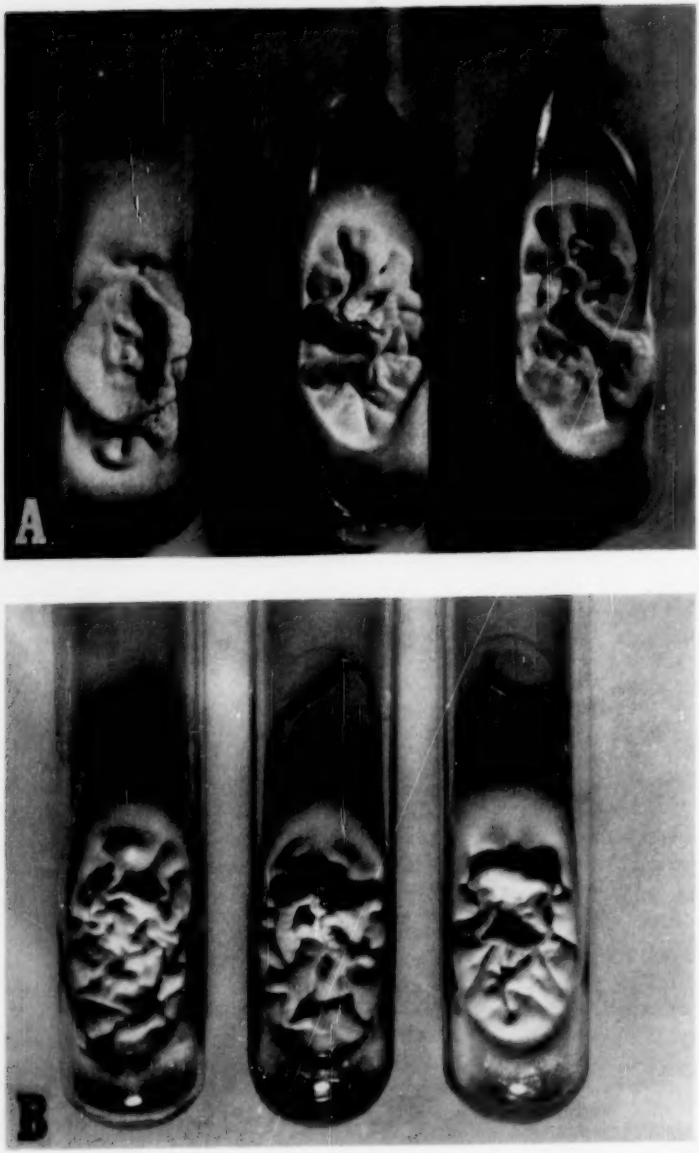

FIG. 1. A–B.

(5) Centraalbureau voor Schimmelcultures (through the kindness of Dr. G. A. de Vries, Baarn, Netherlands)

T. circonvolutum (1 isolate)
T. epilans (1 isolate)
T. areolatum (1 isolate)
T. floriforme (1 isolate)

All cultures were studied on Sabouraud dextrose agar and were described when first isolated or upon receipt from the culture collections. Variety names were assigned to each on the basis of colony configuration, pigmentation, etc., as they approximated the descriptions in the literature for the various species. For example, of the recently isolated strains: 2 were of the crateriform type, 1 was a cerebriform type, 3 were atypical sulfureum types, and 4 appeared to be intermediate types not fitting any of the described species. Of the named cultures, most of the isolates appeared to be characteristic of the "species" they represented. However, all of the isolates labelled *T. sulfureum*, except two, had no trace of the characteristic yellow pigment, but it was assumed that they had once had this pigment or would not have been so classified. Also, four isolates had become partially pleomorphic. Serial transfers, however, with careful selection of powdery areas brought all except three strains into sporulating condition.

Single spore strains were made from the 36 sporulating cultures by selecting isolated microconidia according to a method previously described (**14**). It was felt that the use of single spore cultures would be valuable in comparative morphological studies and in showing variations that might occur in pure strains while undergoing study. Eight spores were selected from each isolate. Usually the colonies that developed from the eight spores appeared similar to each other and to the original culture. In a few cases, however, one or two of the spores would give rise to colonies which appeared to be slightly different from the others in the group. One colony from those which appeared to be characteristic was selected from each group. Each of the single spore strains then was studied to determine its characteristic gross and microscopic morphology. The microscopic morphology will be discussed first.

FIG. 1. A. Monospore strain No. *399*. Recent isolate, Mycology Unit, Communicable Disease Center. Originally was of acuminate type with deep, yellow surface pigment but has formed 3 colonial types after serial transfers over a 2-year period: left to right, crateriform, acuminate, and cerebriform. B. Monospore strain No. *410*. *T. acuminatum* from Dr. A. Catanei. Originally was a typical acuminate type. Three colonial types developed after serial transfers over a 2-year period: left to right, cerebriform, acuminate, crateriform.

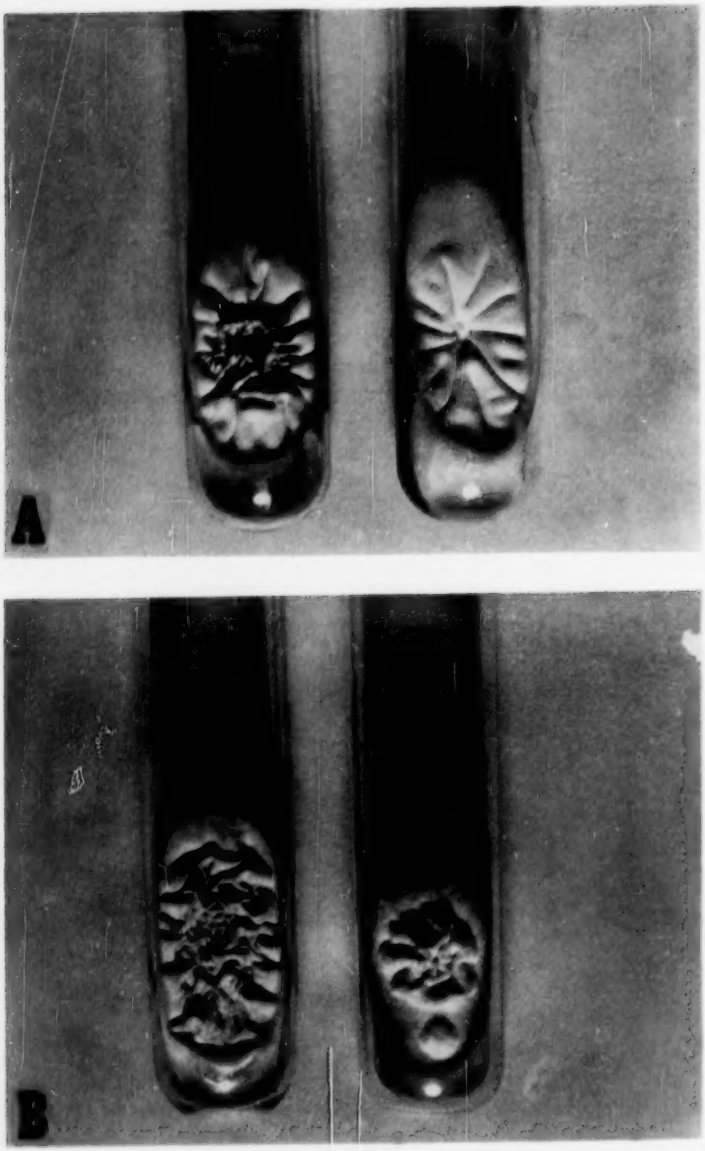

FIG. 2. A–B.

The microscopic morphology of 36 strains studied on a variety of media showed no essential differences and all conformed rather closely to the description given for *T. tonsurans* in Part II of this paper. What variations did occur appeared to be referable to the state of the culture at the time studied. For example, microconidia and macroconidia were most numerous in powdery cultures. Cultures with downy or felt-like surfaces produced fewer spores, and fluffy cultures showed very few microconidia. That the cultures could pass from powdery-surfaced colonies to downy to fluffy forms in any sequence was seen in the long-term study of these single spore strains. In the fluffy cultures the microconidia were extremely thin and delicate, but if by careful selection a powdery culture was obtained the microconidia again appeared typical of the species. Macroconidia were observed in 20 of 36 strains. Their production was greatly stimulated by the use of Wort agar (Difco). Variation in the form of the macroconidium was remarkably great both among the different strains as well as in a single strain. The morphology of these structures is described and illustrated in Part II of this paper. It will be noted that variations within a single strain were as great as those among the 20 strains studied. It was found also that no particular microscopic morphology could be associated with any one colonial type.

Each of the 36 strains was serially transferred on Sabouraud dextrose agar every 10 to 14 days over a period of 2 years, and any variations in colony type were noted. If a variant was obtained, it was held and subcultured, and a new series was started from the parent strain. At the end of the two-year period, 30 Sabouraud dextrose agar slants were planted from each strain as well as from each of the variants that had developed from them.

Results indicated that most of the transfers were identical with the parent cultures, which would indicate considerable stability of the colony types. However, in all cases a number of colonial variants were produced by each of the single spore strains, with the exception of 4 strains which had become pleomorphic early in the study. Some of these variants had become quite stable, although a few did revert after several

FIG. 2. A. Monospore strain No. *397*. *T. sulfureum* from Sabouraud Collection. Originally was typical sulfureum type except that it had a tan surface pigment. It developed 2 colonial types after serial transfers over a 2-year period: left to right, crateriform with tan pigment, sulfureum with bright yellow-orange pigment. B. Monospore strain No. *948*. *T. crateriforme* from Sabouraud Collection. Originally was a typical crateriform type. It has developed 2 colonial types after serial transfers over a 2-year period: left to right, cerebriform, and acuminate.

transplants. The variants developed by single spore strains were distinctive enough to warrant designations as different species on the basis of the descriptions in the literature. For example, a typical crateriform type gave rise to a cerebriform as well as a sulfureum type. A typical sulfureum type gave rise to a cerebriform and a crateriform type. A large number of indeterminate forms were also produced. Some of the variants obtained from single spore strains are illustrated in FIGS. 1, 2.

Physiological studies were also carried out with these isolates. This work has been reported in a previous paper (**42**) and will be reviewed here only briefly, as the findings have some bearing on the taxonomy of *T. tonsurans*. All 36 strains were studied on vitamin-free media and amino acid-free media, to which the various vitamins and amino acids, respectively, had been added. Results indicated that all the members of this series had similar nutritional requirements. One striking characteristic was a partial deficiency for thiamine which could be replaced only by certain pyrimidine compounds. All of the strains had this deficiency which was not found in the dermatophyte species, *T. rubrum* and *T. mentagrophytes*, which were used as controls. This requirement for thiamine for various isolates of the "Crateriform Group" has been reported previously by Mariat and Drouhet (**27**) and by Sullivan, Bereston, and Wood (**41**). These findings give further evidence for the very close relationship of the various colonial types in this group.

It will be noted that in the case of some of the "species" obtained from culture collections, only one strain was available for study. In most instances these cultures fitted so closely into the group that there was no doubt as to their close relationship to the others. In three instances, however, the only available isolate was pleomorphic, or nearly so, and did not conform to the morphological patterns of the others. For example, *T. circonvolutum* developed an apparently pleomorphic, white, fluffy culture, and no spores were produced on any medium studied. Although pleomorphic strains of *T. tonsurans* exhibit little stimulation by thiamine, this strain did show some increase in growth on a thiamine-containing medium. In the case of *T. areolatum*, the culture was again white and fluffy, but a few delicate microconidia could be obtained on wort agar. This strain also showed some stimulation by thiamine. *T. floriforme* was in the form of a white, fluffy culture without spores, and showed a slightly different nutritional pattern. Here the organism showed a deficiency for thiamine, but also was deficient for another vitamin, pyridoxine.

Although morphological identity with the other strains thus could not be demonstrated for these three "species," *T. circonvolutum*, *T.*

areolatum, and *T. floriforme*, it appeared logical to include them as synonyms on the basis of the literature descriptions of these organisms when first isolated, as well as on the fact that two of these exhibited nutritional requirements similar to those of *T. tonsurans*. The different nutritional pattern exhibited by *T. floriforme* might raise doubt as to whether this fungus should be included in the synonymy. In the original description by Beintema (**1**), however, it was clearly shown that it produced an endothrix-type invasion of the hair, and the gross and microscopic morphology of the fungus fell well within the morphological limits of *T. tonsurans*. There would appear to be little reason to place it in the genus *Favotrichophyton* as suggested by Dodge (**10**).

In an attempt to determine whether any particular colonial form of the fungus could be associated with any specific clinical picture, data were obtained, whenever possible, concerning the type of lesion from which the hair specimens had been collected. Results of these investigations showed no correlation between the type of lesion and the colonial variant obtained from it. The sulfureum variety, for example, was isolated from suppurative lesions, dry and scaling lesions, eczematoid lesions, and lesions which resembled lupus erythematosus. This lack of association of a colony type with a consistent clinical pattern has also been noted by Pipkin (**35**) and others in this country.

Type of hair invasion was also studied in relationship to the type of culture isolated. No particular type of endothrix hair was found associated with any one colonial form. For example, long hairs sparsely filled with mycelium, as well as short, thick stubs filled with round spores, occurred in specimens which gave rise to the crateriform-type culture; and, although one type of hair invasion seemed to be characteristic of a single patient, both types could be found often in the same patient if a large number of hairs were examined. It was difficult to evaluate differences in resistance of the mycelium in the hairs to the action of KOH. However, after studying a large number of hairs which produced different colonial forms, no consistent association could be found either between the type of hair invasion or the colony form developed.

SYNONYMY

As a result of these studies and a critical review of the literature, the following list of synonyms for *T. tonsurans* has been compiled:

TRICHOPHYTON TONSURANS Malmsten, 1845 (**26**)

Trichomyces tonsurans Malmsten, 1845 (nomen nudum) (**26**)
Oidium tonsurans (Malmsten) Zopf, 1890 (**46**)
Trichophyton sabouraudi Blanchard, 1896 (**3**)
Trichophyton crateriforme Bodin, 1902 (**4**)
Trichophyton acuminatum Bodin, 1902 (**4**)
Trichophyton regulare Sabouraud apud Favera, 1909 (**11**)
Trichophyton fumatum Sabouraud apud Favera, 1909 (**11**)
Trichophyton exsiccatum Sabouraud, 1910 (**40**)
Trichophyton polygonum Sabouraud, 1910 (**40**)
Trichophyton cerebriforme Sabouraud, 1910 (**40**)
Trichophyton circonvolutum Sabouraud, 1910 (**40**)
Trichophyton effractum Sabouraud, 1910 (**40**)
Trichophyton umbilicatum Sabouraud, 1910 (**40**)
Trichophyton pilosum Sabouraud, 1910 (**40**)
Trichophyton plicatile Sabouraud, 1910 (**40**)
Trichophyton sulfureum Sabouraud, 1910 (**40**)
Neotrichophyton plicatile (Sabouraud) Castellani & Chalmers, 1919 (**8**)
Neotrichophyton flavum (Bodin) Castellani & Chalmers, 1919 (**8**)
Trichophyton ochropyrraceum Muijs apud Papegaaij, 1924 (**33**)
Trichophyton rotundum MacCarthy, 1925 (**25**)
Chlamydoaleurosporia crateriformis (Bodin) Grigoraki, 1925 (**19**)
Aleurosporia effracta (Sabouraud) Grigoraki, 1925 (**19**)
Aleurosporia acuminata (Bodin) Grigoraki, 1925 (**19**)
Aleurosporia plicatilis (Sabouraud) Grigoraki, 1925 (**19**)
Trichophyton fuscum sulcatum Neuber, 1925 (**31**)
Trichophyton rotundum MacCarthy, 1925 (**25**)
Trichophyton (*Aleurosporia*) *effractum* (Sabouraud) Guiart & Grigoraki, 1928 (**21**)
Trichophyton (*Aleurosporia*) *plicatile* (Sabouraud) Guiart & Grigoraki, 1928 (**21**)
Trichophyton (*Aleurosporia*) *acuminatum* (Bodin) Guiart & Grigoraki, 1928 (**21**)
Trichophyton (*Chlamydoaleurosporia*) *umbilicatum* (Sabouraud) Guirat & Grigoraki, 1928 (**21**)
Trichophyton (*Chlamydoaleurosporia*) *crateriforme* (Bodin) Guiart & Grigoraki, 1928 (**21**)
Trichophyton (*Chlamydoaleurosporia*) *fumatum* (Sabouraud) Guiart & Grigoraki, 1928 (**21**)
Trichophyton bicolor Veiga, 1929 (**45**)
Trichophyton cineraceum Veiga, 1929 (**45**)
Trichophyton acutulum Veiga, 1929 (**45**)
Trichophyton arcolatum Negroni, 1929 (**30**)
Trichophyton germen I. Gregorio, 1931 (**18**)
Trichophyton floriforme Beintema, 1934 (**1**)
Favotrichophyton floriforme (Beintema) Dodge, 1935 (**10**)
Trichophyton fuscum Dodge, 1935 (**10**)
Endothrix crateriforme (Bodin) Orbtel, 1950 (**32**)
Endothrix acuminatum (Bodin) Orbtel, 1950 (**32**)
Endothrix sulfurcum (Sabouraud) Orbtel, 1950 (**32**)

VERNACULAR DESIGNATIONS:

The following are designations which undoubtedly also refer to *T. tonsurans*, but cannot be included in the synonymy because they are not binomials.

Trichophyton mégalosporon endothrix Sabouraud, 1894 (**39**)
Trichophyton à culture acuminée Sabouraud, 1894 (**39**)
Trichophyton à culture crateriforme Sabouraud, 1894 (**39**)
Trichophyton with the primrose crater Fox, 1908 (**13**)

DOUBTFUL SPECIES:

The following are species often included in this group, but are either *nomina ambigua*, or are so poorly described that they cannot be placed in the above synonymy.

Trichophyton depilans Mégnin, 1878 (**29**)
Trichophyton epilans Boucher & Mégnin, 1887 (**5**)
Trichophyton flavum Bodin, 1902 (**4**)
Trichophyton blanchardi Castellani, 1908 (**7**)
Trichophyton cerebriforme Sabouraud, 1910 (**40**)
Trichophyton spongoides Fiocco & Minassian apud Fiocco, 1910 (**12**)
Trichophyton inflatum Fiocco & Minassian apud Fiocco, 1910 (**12**)
Trichophyton currii Chalmers & Marshall, 1914 (**9**)
Trichophyton sulfuratum Bessunger, 1915 (**2**)

The following vernacular designation should also be placed in this group as it is doubtful that it refers to *T. tonsurans:*

Trichophyton à culture jaune, craquelée, vermiculaire Sabouraud, 1893 (**38**)

It is suggested that if it is desirable to indicate the particular morphology of a strain when isolated, this be indicated in the variety name: for example, *T. tonsurans* var. *crateriforme*, or *T. tonsurans* var. *sulfureum*.

SUMMARY

The history and taxonomy of *Trichophyton tonsurans* have been presented. Morphological studies of single spore strains of 13 recent isolates as well as of 12 named "species" have shown a varied morphological picture with regard both to colony form and microscopic structures. Serial transfers of these cultures over a two-year period have indicated, however, that none of these characteristics are stable and occur at random among the isolates. Thus the cultures studied can be considered as unstable morphological variants of a single species.

Nutritional studies of these fungi, presented in detail in an earlier paper (**42**), had shown that there is also a close physiological similarity among these strains. All of the isolates possessed a similar partial

deficiency for thiamine which gives further evidence for their identity. One strain required pyridoxine as well.

It is interesting to note that hyphal fusion studies made with several members of this group by Taschdjian and Muskatblit (**43**) give further support to the hypothesis that many of the "species" described in the literature are synonyms of *T. tonsurans*.

Practical applications of these morphological and physiological studies in the laboratory identification of *T. tonsurans* are discussed in Part II of this paper.

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UNDESCRIBED SPECIES OF SYNCHYTRIUM [1]

JOHN S. KARLING

(WITH 20 FIGURES)

Species of *Synchytrium* are widely distributed in the western hemisphere and parasitize species of most orders and families of flowering plants. This is shown by the large number of species described in the literature as well as by the numerous specimens available in herbaria in the United States and elsewhere. In studying the collections in these herbaria, the author (**1955a**, **b**, **c**) found several named and unnamed species which have not been described or diagnosed in the literature on *Synchytrium*. Additional species have come to light in recent months, and a careful study has been made of them with the objective of determining their identity and relationship to older and better known species. Accordingly, the present contribution and others to come concern the morphology, classification, types of galls induced and other characteristics of such species.

Obviously, herbarium specimens are not favorable for study of life cycles and types of development. However, if the infected host is properly treated, sectioned and stained, the sections may reveal certain key stages and structures of the fungi which make identification and classification possible. This became evident to the author (**1955a**) in a study of Spegazzini's species, some of which were collected almost three-quarters of a century ago. Prosori, for example, were recognizable in several species as empty collapsed vesicles in the infected cells, which made it possible to assign such species to certain subgenera. Structures such as prosori are almost impossible to find and recognize by dissection of dried herbarium material, and it is essential that cytological techniques be used to study such specimens. In the present investigation, portions of the infected host were thoroughly soaked in water, fixed in killing solution in a vacuum to remove air, embedded in paraffin, sectioned at 7 μ and stained in the usual manner. Although

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the host tissues in such sections are usually shrunken, the fungus, particularly its resting spore stages, may be so well preserved that its nuclei are still recognizable.

SYNCHYTRIUM ON POLEMONIUM

This species was collected by W. N. Suksdorf on *Polemonium occidentale* on Mt. Adams, Yakima Co., Washington, September 17, 1894, according to herbarium specimens kindly loaned to the author by Dr. C. G. Shaw of the State College of Washington. Suksdorf apparently sent it to Ellis for identification and naming, and later it was distributed by Ellis and Everhart as *S. polemonii*. Cook (**1951**), Cooke and Shaw (**1953**), and Cash (**1954**) listed it among the species of *Synchytrium* but did not describe it. Only resting spores (FIG. 2) and their galls (FIG. 1) are present in Suksdorf's material, but since it was collected in the fall, it is possible that this species may produce sporangial sori in the spring and summer months. However, until this stage has been found, it is regarded by the author as a short-cycled species, and on the basis of the present study it may be diagnosed as follows:

Synchytrium polemonii Ell. & Everh. sp. nov.

Sporis perdurantibus singularibus, hospitem cellulam implentibus, subsphaericis, 120–160 μ diam., vel ovalibus, 126–140 × 150–165 μ diam.; exosporio levi, 3.2–2.8 μ crasso, endosporio hyalino, 2–2.6 μ crasso, contentu luteo, granulari; germinatione ignota.

Specimen typicum in Herb. State Coll. Wash. conservatum; Suksdorf n. *417*.

Resting spores usually solitary and completely filling host cell, spherical, 120–160 μ, or oval, 126–140 × 150–165 μ, with a light-amber, 3.2–3.8 μ thick, smooth exospore and a hyaline, 2–2.4 μ endospore; content coarsely granular and dark yellow; residue usually absent; germination unknown.

Compositely monogallic, resting spore galls abundant on both surfaces of leaves and on petioles, single and scattered or aggregated and confluent, protruding prominently, 168–266 μ high by 210–380 μ broad at base, narrowly to broadly crateriform at apex; sheath around infected cell usually 4–6 cells thick.

On leaves and petioles of *Polemonium occidentale*, Yakima Co., Washington, U. S. A., causing local thickening, crinkling and distortion of young leaves.

So far this is the first species to be described on a member of the family Polemoniaceae. The sizes and shapes of the resting spores fall within the range of variations reported for *S. aureum*, which is said to

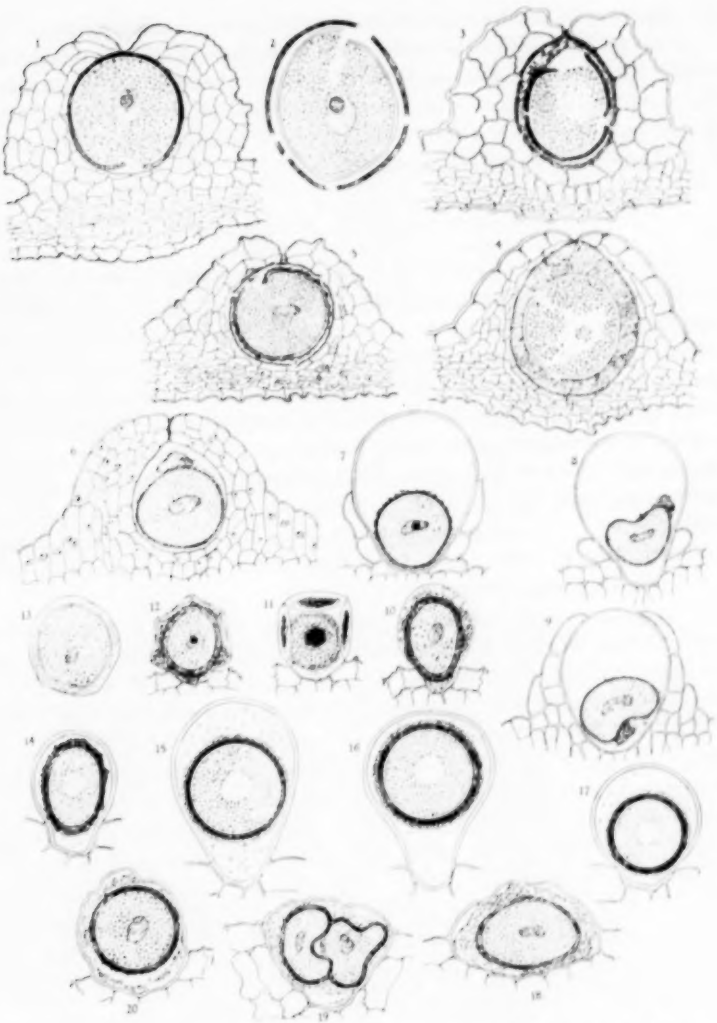

FIG. 1. Resting spore gall, *Synchytrium polemonii*. 2. Large resting spore with contracted endospore and contents, *S. polemonii*. 3. Resting spore gall, *S. nitidum*. 4. Resting spore gall of *S. plantagineum* (?) on *Plantago lanceolata*. 5. Resting spore gall of *S. plantagineum* (?) on *Plantago tweedyi*. 6–9. Resting spore galls of *Synchytrium* spp. on *Sibbaldia procumbens*. 10–12. Resting spore galls, *S. namae*. 13. Thick-walled resting spore of *S. namae* with contracted endospore and contents. 14–17. Variations in resting spore galls of *Synchytrium* sp. on *Phlox gracilis*. 18–20. Resting spore galls of *S. echii*.

occur on several hundred species in 33 families of flowering plants. The most conspicuous difference to be noted is the lack of residue around the resting spore of *S. polemonii* (FIG. 1) and its abundance in *S. aureum*. However, this difference is an expression of host reaction, and until both species have been grown on the same hosts, it is impossible to determine how significant this difference may be.

SYNCHYTRIUM ON SPECULARIA

This species was collected by H. S. Jennings on *Specularia perfoliata* at College Station, Texas, Apr. 24, 1890. According to Cash (**1954**), Jennings apparently sent it to Ellis for identification, and as far as the author knows it has never been described in the literature. Herbarium specimens were secured from the U. S. Dept. of Agric., through the courtesy of Dr. John A. Stevenson, and accompanying this material is a note on *S. nitidum* which presumably was written by Ellis: "Galls thickly scattered over both sides of leaf, hemispheric-pustuliform, minute, shining purplish black. Each containing a single large (150–200 μ) resting spore." In examining the specimens the author found an abundance of black pycnidia of a fungus imperfectus on the leaves and only few scattered *Synchytrium* galls.

The galls contain only resting spores, and inasmuch as the material was collected in April in Texas when sporangial sori would probably be present in a long-cycled species, it is quite possible that *S. nitidum* is a short-cycled species which belongs in the subgenus *Pycnochytrium*. The few galls in the material at hand are usually single and scattered, or rarely aggregate and confluent, composite and multicellular, narrowly crateriform at the apex, and protrude conspicuously, as shown in FIG. 3. The sheath is 2 to 3 cells thick, and its cells appear to be markedly enlarged. The walls of the epidermal cells around the apex and sides of the gall may be up to 7.2 μ thick. Also, the wall of the infected cell is quite thick, 4–5 μ, as shown in FIG. 3. The resting spores are solitary, subspherical to oval in shape and almost fill the infected cell, and the residue around them is usually sparse. On the basis of this limited information *S. nitidum* may be diagnosed as follows:

Synchytrium nitidum Ell. & Gall. sp. nov.

Sporis perdurantibus solitaris, subsphaericis, 108–114 μ diam., vel ovalibus, 92–106 × 112–120 μ diam., parietibus levibus, 3.8–4.2 μ, crassis, contentu granulari; germinatione ignota.

Specimen typicum in Herb. N. Y. Bot. Gard. conservatum; Jennings n. *110*.

Resting spores usually solitary, filling host cell almost completely, subspherical, 108–114 μ, or oval, 92–106 × 112–120 μ, with a 3.8–4.2 μ thick, smooth wall and coarsely granular content; enveloping residue sparse; germination unknown.

Compositely monogallic, resting spore galls usually single and scattered, rarely aggregated and confluent, protruding, usually narrowly crateriform at apex with base embedded in the host tissue, 228–300 μ broad by 258–280 μ high, with a sheath 2–3 cells thick.

On the leaves and stem of *Specularia perfoliata*, College Station, Texas, and *S. biflora*, Baton Rouge, La.

This is the first species to be described on *Specularia*, but other species, *S. globosum* and *S. aureum*, have been reported on *Campanula*, another genus of the family Campanulaceae. Like *S. nitidum*, both of these are short-cycled species and form only resting spores, but whether *S. nitidum* is closely related or identical to either of them will not be certain until it has been studied more intensively from living material. In this connection it may be noted that Dr. C. A. Brown has collected *S. nitidum* on *S. biflora* at Baton Rouge, La., but the author found only a few resting spore galls and spores in this material.

SYNCHYTRIUM PLANTAGINEUM SACC. & SPEG.

This species was established and described by Saccardo and Spegazzini (in Saccardo, **1878**, p. 243) for a parasite on leaves of *Plantago lanceolata* in Italy. The description is repeated on the labels of the exsiccati, distributed as Myc. Ven. No. *1125*. The authors diagnosed it as follows: "Tuberculis amphigenis hinc inde dense gregariis, granuliformibus, initio ochraceo-rufis, dein nigricantibus, sporangiis?) sphaericis 110–130 μ diam., flavo-ochraceis, anhisto-tunicatis, guttulis oleosis flavicantibus." Obviously, their description of the fungus is too brief to determine its validity and relationship, and the status of *S. plantagineum* is questionable. The size of the reported sporangia is unusually large, and for this reason Tobler (**1912**) regarded them as sporangial sori. The author believes they are resting spores. According to herbarium specimens at the Universities of Illinois and Nebraska, Tracy (**1888**) and Tracy and Earle (**1895**) collected what they believed to be the same species on the same host at Starkville, Miss. Overholts (**1933**) and Maneval (**1936**) also reported it on *P. lanceolata* in Missouri, but none of these collectors described it further.

The author has been unable to secure type material of this species for examination, but he has made a careful study of Tracy's species from Mississippi to determine if it is the same one described by Saccardo and

Spegazzini. Only resting spores and their galls are present in this material. The resting spores are solitary, buff or tan in color, spherical, 90–172 μ, or oval, 115–140 × 160–170 μ, with a smooth, 4–6 μ, thick wall and coarsely granular content. As shown in FIG. 4 they are enveloped by a 10–22 μ thick, dense and almost homogeneous layer of residue which may fill the remainder of the host cell and is usually cracked by almost radial lines. The galls are usually single and scattered on both surfaces of the leaves, occasionally aggregated and confluent, protruding, subhemispherical or broadly dome-shaped, 198–288 high by 240–488 μ broad, and usually narrowly crateriform at the apex. The base is embedded in the leaf tissue and frequently causes the opposite side of the leaf to protrude (FIG. 4). All galls are composite and multicellular with a sheath 3 to 6 cells thick. The outer walls of the epidermal cells around the protruding portion of the gall are up to 8 μ thick, and the other sheath cells are markedly enlarged but thin-walled. However, because of their thin walls they were collapsed in the herbarium material, and it is impossible to determine their relative size. It is apparent that the galls in Tracy's specimens are quite large, whereas in Saccardo's and Spegazzini's material they were reported to be granule-like and confluent. Also, the resting spores in Tracy's material are considerably larger.

Other so-called species of *Synchytrium* have been collected several times on *Plantago*, but it is not certain that all of these are one species. *Synchytrium aureum* has been reported on *P. psylli* in Italy (Passerini, **1881**), *P. lanceolata* and *P. major* in Germany (Schroeter, **1885**), *P. alpina* in Switzerland (Rytz, **1907, 1932**), *P. virginica* in Chile (Neger, **1908**) and *P. major* in Sweden (Hammarlund, **1932**). Prior to these reports Sorokin (**1877**) described *S. punctum* on *P. media* in Russia. In 1919 Spegazzini collected a species on *P. media* (?) at La Plata, Argentina, which he labeled *S. plantaginicola*. In a study of his type material the author (**1955a**) found only resting spores and their galls. The resting spores in this material are usually solitary, occasionally 2 in a cell, oval, 59–72 × 88–105 μ, or subspherical, 115–156 μ, with a 2.9–3.6 μ thick, smooth brown wall, pale-yellow to hyaline, coarsely granular content, and are enveloped usually by a 3–12 μ thick layer of residue. The galls occur on both surfaces of the leaves, singly and scattered, or aggregated and confluent and rarely compound. They are also predominantly composite, 192–640 μ diam. at base by 140–400 μ high, and rarely simple. Accordingly, the sizes of the galls approach closely those in Tracy's specimens. Furthermore, the walls around the protruding portions of the gall are up to 10 μ thick, and the other sheath cells may

be 4 to 5 times as large as normal cells of the host. Thus, with respect to size, shape, and shape of the galls, Spegazzini's *S. plantaginicola* induces very similar local host reactions to those caused by the species collected by Tracy. Spegazzini, however, did not describe his species, and he apparently regarded it as different from the one he and Saccardo collected in Italy. Sorokin's *S. punctum* on *P. media* apparently causes only simple galls, develops very small, 7–20 μ, resting spores, and as far as is known, it appears to be different from any of the known species on *Plantago*.

Recently, another species was collected on *Plantago tweedyi* during a mycological foray in Wyoming and listed as an unidentified species by Raper and Cooke (**1954**). Material of this species was generously contributed by W. G. Solheim of the University of Wyoming. Only resting spores and their galls were found in this material. The galls occur on both surfaces of the leaves and are usually single and scattered or they may be aggregated and confluent. They are composite and multicellular, 132–150 μ high by 216–264 μ broad at the base, and narrowly crateriform at the apex with a sheath 2 to 4 cells in thickness, as shown in FIG. 5. The epidermal cells at the apex and around the protruding portion of the gall are slightly elongate and enlarged, but their walls are not thickened or lignified. The other sheath cells are enlarged also, but in the sections of the herbarium material they are so shrunken that their relative sizes cannot be determined. The resting spores are subspherical, 70–90 μ, or oval, 60–72 × 90–102 μ, with a 3.6–4.2 μ thick, smooth wall and coarsely granular content. They fill the host cell completely or almost so, and the residue around them is very sparse (FIG. 5), or it may be lacking entirely. Accordingly, the galls induced by this species and its resting spores are considerably smaller than those of Tracy's and Spegazzini's fungi, and in these respects it resembles more closely Saccardo's and Spegazzini's Italian species.

As far as they are known, all of the species reported on *Plantago* are short-cycled and appear to belong in the subgenus *Pycnochytrium*. Except for *S. punctum*, they may possibly be one species, and in that event they may be synonymous with *S. aureum*. The sizes, shapes and structure of their galls and resting spores fall within the range of variations reported for *S. aureum* on its various hosts. However, it should be emphasized that all of the reports of the occurrence of *S. aureum* may not relate to one species because of the tendency of many mycologists to give this name to any short-cycled species with yellow or orange color whose resting spores are borne in composite galls.

SYNCHYTRIUM SPP. ON SIBBALDIA PROCUMBENS

A species of *Synchytrium* on *Sibbaldia procumbens* was listed by Raper and Cooke (**1954**) in their report of the mycological foray in Wyoming. In examining infected plants of this host collected by Dr. C. G. Shaw, August, 1950, in Wyoming, the author found what may possibly be two distinct species of *Synchytrium*. The most common one induces the development of dome-shaped or almost hemispherical, brilliant, dark-lavender-red composite, narrowly crateriform, 108–192 μ broad by 58–168 μ high, resting spore galls (FIG. 6) with a sheath 3–6 cells thick. These occur singly and scattered but are usually aggregated and frequently confluent on both surfaces of leaves and on petioles. In longitudinal sections, such galls sometimes seem to "sit" on the palisade or mesophyll parenchyma, but in most galls the lower part is embedded in these tissues. In the latter cases the mesophyll or palisade cells are quite probably stimulated to divide like the epidermal cells, so that the galls are composed of the enlarged infected cells and derivatives of the epidermal and parenchyma cells. In some galls, on the other hand, the sheath appears to have been derived almost exclusively from the epidermis. That infection has a marked influence on the epidermal cells is quite evident in all galls. Normally, these cells are horizontally elongate, 20–38 × 10–14 μ, but in the vicinity of isolated galls or between two fairly close galls they are elongated vertically or outward and very narrow, meristematic in appearance, and thin-walled, which indicates (FIG. 6) that several transverse divisions of the original cells have occurred. Usually, the epidermis in such regions has a distinct palisade arrangement, and the thin-walled cells which compose it are often transversely divided.

Whether or not these are resting spore galls is not absolutely certain because what the author interprets as resting spores within them are not very thick-walled and do not contain the usual coarsely granular protoplasm. Instead, it is usually densely alveolar in appearance like that of sori or prosori. This may possibly be due to immaturity of these thalli or to the fixative used, but the author is regarding these bodies as resting spores for the time being. They are oval, 44–59 × 58–72 μ, or subspherical, 42–57 μ, in shape, with a 1.8–2.8 μ thick, smooth wall. They fill the infected cell only partly and are enveloped by a sparse to a fair amount of residue in which the degenerating host nucleus lies (FIG. 6).

No sporangia were found in Shaw's material, and from present data this species seems to be short-cycled and to belong in the subgenus *Pycnochytrium*. However, since the material was collected in late

summer, when the sporangial cycle of most *Synchytrium* species has been completed, it is possible that it may develop sori and sporangia earlier in the year and is a long-cycled species. Further study at different seasons of the year are necessary for solving this problem. Its so-called resting spores are considerably smaller than those of *S. aureum*, and it does not appear to be identical with the latter species.

The second species found on *Sibbaldia procumbens* induces galls which are quite unlike those of the previous one in appearance, and seem to represent a different species. These galls are composed largely of a greatly enlarged vesicular epidermal cell which protrudes conspicuously on the surface of the host as shown in FIGS. 7–9. These may occur singly and scattered or are aggregated on both surfaces of leaves and on petioles, but they are not often intermingled with galls of the previous species. The galls of the two species usually occur separately, but occasionally a few may be intermingled, and in such instances the differences in appearance are quite striking. When the galls of the second species dry out, the apex usually invaginates so that they appear cup-shaped like the dried galls of *S. potentillae*. For this reason the species was first regarded as *S. potentillae*, or a variety of it, but a study of longitudinal sections shows that the lower half or base of the infected cells is enveloped by a fringe or sheath of raised and sometimes divided epidermal cells. Therefore, the galls are composite instead of simple and unicellular as has been reported for *S. potentillae*. The sheath is almost always one cell in thickness, rarely 2-celled at the base, and usually extends almost to the equator of the infected cell (FIG. 7). Sometimes, however, it is confined to a circular row of single cells around the base as shown in FIG. 8, and in a few galls it extends almost to the apex (FIG. 9). In the latter galls the epidermal cells apparently have divided one or more times. Sometimes the galls appear to "sit" on the palisade or mesophyll parenchyma (FIG. 7), but in most cases the tapering base of the infected cell extends down into these tissues (FIGS. 8, 9). However, it is apparent that the reduced sheath is derived from the epidermis, and that the entire gall is composed entirely of enlarged, divided and elongate epidermal cells. These galls are predominantly obpyriform in shape, 78–150 μ in broadest diameter and 84–138 μ high, and brilliantly lavender-red in color. The wall of the infected cell is 2.8–3.2 μ thick and orange- to reddish-brown in color.

So far only resting spores have been found in these galls, and they usually lie in the base. They are yellowish-orange to orange in color, oval, 54–66 × 46–52 μ, or subspherical, 42–57 μ, with a 1.8–2.9 μ thick, smooth wall and densely alveolar content, and fill only a small portion

of the infected cell. In longitudinal sections of the dried galls they are usually collapsed and irregular in shape (FIGS. 8, 9). The amount of residue around them is usually sparse, and the degenerating host nucleus may occur above or below the spores (FIGS. 8, 9). These spores are very similar to those of the other species on *Sibbaldia procumbens* in appearance, content, structure and size. However, the two species differ in their effect on the host. Outside of causing marked enlargement of the infected cell the second species causes but little reaction in the adjacent healthy cells, whereas in the first one the epidermal cells are affected for a considerable distance from the galls (FIG. 6). Also, the first species causes greater proliferation of the healthy cells whereby a thick sheath is developed around the infected cell. Accordingly, the two species cause different reactions in the same host, and on this basis they appear to be different species.

SYNCHYTRIUM ON NAMA HISPIDUM

Synchytrium namae sp. nov.

Sporis perdurantibus sphaericis, 36–78 μ diam., vel ovalibus, 24–50 × 32–72 μ diam., parietibus levibus, 5–8.5 μ crasso, contentu granularii; germinatione ignota. Specimen typicum in Herb. State Coll. Wash. conservatum; Shaw n. *36109*.

Resting spores usually solitary, occasionally 2 in a cell, spherical, 36–78 μ, or oval, 24–50 × 32–72 μ, with a smooth, 5–8.5 μ thick, layered wall, and coarsely granular content; usually enveloped by a dense homogeneous, reddish-brown layer of residue which may fill remainder of host cell; germination unknown.

Simply monogallic, resting spore galls numerous, separate or aggregated, oval, 48–65 × 52–84 μ, oblong, 52–60 × 80–90 μ, obpyriform, 36–38 × 60–72 μ, or almost spherical, 38–65 μ, with a thin hyaline wall.

On leaves and petioles of *Nama hispidum*, Starr Co., Texas, USA.

The host of this species was collected by Drs. L. Constance and C. L. Lundell in a desert scrub 10 miles west of the Rio Grande River in Starr Co., Texas, and in examining the distributed herbarium specimens of this plant Dr. C. G. Shaw found some of its leaves to be infected by a species of *Synchytrium* which he generously sent to the author. As shown in FIGS. 10–12, the simple unicellular galls induced by *S. namae* are smaller and generally more globular than those caused by other simply monogallic members of the subgenus *Pycnochytrium*. In dried material the galls and their resting spores look like minute shriveled reddish-brown berries (FIG. 12). The resting spores (FIG. 13) have an unusually thick, layered wall and are enveloped by a dense

layer of residue which usually fills the remainder of the host cell completely. It is this residue and not the hyaline wall of the infected cell which gives the galls their characteristic color.

The galls are the result of enlargement of the infected epidermal cells, and the adjacent healthy epidermal, palisade and mesophyll cells are not markedly affected. In the case of clavate galls the tapering base may extend slightly downward among the palisade or mesophyll cells (FIG. 10). Gall size, shape, and structure are obviously expressions of host reaction, and it is possible that the galls caused by S. *namae* may be found to vary considerably when this species is grown on other hosts. Therefore, too much value should not be placed on this character alone. However, the small size of the resting spores with their unusually thick walls together with the minute size of the galls distinguish it from other species, in the author's opinion, and he is describing it as a new species. Possibly, it may be a long-cycled species, but until summer sporangial stages and germination of the resting spores have been observed it is placed provisionally in the subgenus *Pycnochytrium*. So far, it is the first and only species to be reported on a member of the family Hydrophyllaceae.

SYNCHYTRIUM SP. ON PHLOX GRACILIS

The host of this species was collected by W. B. Anderson at Darcy, British Columbia, May 14, 1918, and in examining the herbarium specimens at the State College of Washington, Dr. C. G. Shaw found a few leaves to be parasitized by a species of *Synchytrium* which he kindly sent to the author. Only resting spores occur in this material, and on this basis the species appears to be short-cycled and to belong in the subgenus *Pycnochytrium*. It is characterized by subspherical, 56–144 μ, ovoid, 50–72 $\times$ 60–80 μ, resting spores with a 4–6 μ thick, smooth, reddish-brown wall and coarsely granular content (FIGS. 14–17). These usually occur singly but occasionally 2 and 3 may be found in an infected cell. Their wall is usually enveloped by a dense layer of reddish-brown residue, and the remainder of the infected cell may be filled with granular, almost hyaline residue.

This species induces the development of simple, unicellular galls which consist only of a greatly enlarged, infected epidermal cell, and in this respect it is a simply monogallic species. The galls are predominantly broadly obpyriform or broadly clavate in shape, 138–174 μ in broadest diameter by 98–222 μ high (FIGS. 15, 16). Occasionally oval, 8–120 $\times$ 98–150 μ, and subspherical 160–192 μ, galls occur (FIGS. 14, 17). The wall of the galls is hyaline and smooth, and may be up to 9 μ

thick. No protuberances, openings, or other modifications of the wall have been observed.

According to the author's (**1955d**) classification and with the addition of *S. namae* and *S. echii* there are 14 other simply monogallic species in the subgenus *Pycnochytrium*. Of these *S. selaginellae, S. pyriforme, S. musicola, S. echii, S. nycthantidis, S. ulmariae* and *S. namae* have smaller resting spores than the present species on *Phlox*. The latter's resting spore falls within the reported size range of those of *S. myosotidis, S. potentillae, S. rubrocinctum, S. athyrii* and *S. rhyncosiae*. The size and shape of its galls are somewhat similar to those of *S. musicola, S. rubrocinctum, S. nycthanthidis,* and *S. myosotidis,* but smaller than those of *S. athyrii, S. potentillae* and *S. rhyncosiae*. Because of this overlapping of spore and gall sizes it is very difficult to determine whether or not the fungus on *Phlox* is a new species, and this problem is further complicated by the fact that many of the simply monogallic species are not fully known. Therefore, it is unwise at present to give this species a specific name.

SYNCHYTRIUM ECHII SPEG.

In a previous publication (**1955a**) the author reported that he was unable to find this species in Spegazzini's material, but a more thorough examination since that time revealed a few galls with resting spores on a small area of one leaf. Spegazzini (**1909**) described this species on *Echium plantagineum* in Argentina as follows: "Maculae amphigenae obsoletae indefinitae pallescentes; verruculae hypophyllae sparsae v. hinc inde gregariae non raro confluentes, subsuperficiales subhemisphericae (250 μ dm.) papillulosae, e cellulis epidermicis hypertrophicis ellipticis efformatae testaceae; sporae perdurantes, tantum adhuc notae, in cellulis solitariae globosiae primo hyalinae (18–40 μ dm.) serius lateritiae (40–70 μ dm.) dense minuteque verruculosae, guttula maxima deosa flava foetae."

The present observations confirm the validity of this fungus as a species of *Synchytrium*. Only resting spores were observed in the material studied. These usually occurred singly, but occasionally two were found in the infected cell (FIG. 19). They were spherical, 39–50 μ, ovoid or ellipsoidal, 40–48 $\times$ 48–66 μ, with a smooth, brown, 3–3.8 μ thick wall and coarsely granular content (FIGS. 18, 20). In embedded and sectioned material they were frequently collapsed and invaginated as shown in FIG. 19. In most galls they were enveloped by a dense layer of residue which filled the remainder of the host cell. The present

measurements of the spores conform in general with those of Spegazzini, but the author did not find that the spore wall is thickly and minutely verrucose as described by Spegazzini. In fixed and stained sections in which the enveloping residue had been torn off, the wall was smooth, and it is quite possible that the reported verrucose character of the wall is due to the closely adherent residue (FIG. 20).

Spegazzini described the galls as being subhemispheric and 250 μ in diameter, but in the material studied by the author they were smaller and oval, 48–72 × 65–84 μ, subspherical, 60–85 μ, or broadly pyriform in shape. However, most of them were markedly shrunken, and it is quite probable they are larger in the living condition. They may be single and scattered or aggregated and usually protrude on the surface of the leaf. Occasionally, however, the oval galls (FIG. 18) are not very conspicuous, and sometimes the basal portion extends down between the palisade cells to the mesophyll (FIG. 19). In the small amount of material examined by the author the galls were simple and consisted of a single enlarged epidermal cell with a fairly thick wall (FIGS. 18–20). If this proves to be true of all galls, *S. echii* is simply monogallic in relation to its host. Spegazzini placed this species in the subgenus *Pycnochytrium* under the subgroup *Chrysochytrium*, and the author has found no evidence to justify a change in this classification.

SYNCHYTRIUM LIGUSTICII ON LIGUSTICUM GRAYII

This fungus was collected by W. N. Suksdorf on Mt. Adams, Wash., Sept. 17, 1894, and sent to Ellis who named it *S. ligusticii*. Up to the present time it has been listed under this binomial (Cash, **1954**), but examination of type material at the State College of Washington by the author has shown it to be a species of *Physoderma* which resembles *P. pluriannulata* very closely and is probably identical to it. Therefore, *S. ligusticii* should be excluded from *Synchytrium*.

ADDITIONAL HOSTS OF SYNCHYTRIUM MYOSOTIDIS

Synchytrium myosotidis has been reported on *Myosotis scorpioides*, *M. stricta*, *M. sylvatica*, *Echinospermum lappula*, *Eritrichium* sp., *Lithospermum* sp., *L. arvense* and *Rochelia stellulata*. However, in examining the herbarium material from the State College of Washington the author found several unrecorded hosts of this species which extend its range considerably. With the exception of *Cryptantha angustifolia*, *C. micrantha* and *Pectocarya linearis* these new hosts were recognized as such for the first time by Dr. C. G. Shaw, who in examining the

herbarium specimens for fungi found some of the leaves to be infected by *S. myosotidis*. Inasmuch as these records are new the author is presenting herewith the names of the hosts and their collectors, localities, and dates of collection. Also, a description of the resting spores and the galls is included to show the variations of the fungus on the different hosts.

On *Cryptantha angustifolia*. Leg. et det D. Griffiths, Tucson, Ariz., Feb. 1901. Resting spores up to 5 in a cell, spherical, 80–97 μ, oval, 56–84 × 78–96 μ, occasionally oblong, 48–55 × 75–86 μ, with a 3–3.8 μ thick, smooth, reddish-brown wall and coarsely granular content; enveloped by a small amount of crumbly reddish brown residue. Galls oval, 160–170 μ high by 120–132 μ broad, broadly pyriform, 174–190 × 200–238 μ, or occasionally oblong, 114–125 × 200–216 μ, with a smooth hyaline, 4–5 μ thick wall; closely aggregated along midrib.

On *Cryptantha micrantha*. Leg. et det. D. Griffiths, Tucson, Ariz., Feb. 1901; Leg. C. B. Wolf, Palm Canyon, San Diego Co., Calif., Apr. 7, 1937; det. C. G. Shaw. Resting spores up to 3 in a cell, spherical, 72–128 μ, oval, 60–90 × 76–108 μ, with a reddish-brown, smooth, 5–6 μ thick, wall and coarsely granular content. Galls similar to those on *C. angustifolia*.

On *Cryptantha torreyana*. Leg. A. A. Heller, Colusa Co., Calif., May 6, 1940; det. C. G. Shaw. Resting spores spherical, 67–122 μ, oval, 70–76 × 82–86 μ, with a reddish-brown, smooth 4–5 μ thick wall and coarsely granular content. Galls similar to those on *C. angustifolia*.

On *Pectocarya linearis*. Leg. et det. G. Griffiths, Tucson, Ariz., Feb. 1901. Resting spores up to 3 in a cell, spherical, 88–122 μ, oval, 74–80 × 82–96 μ, with a reddish-brown, 4–5 μ thick smooth wall and coarsely granular content; enveloped by a crumbly layer of residue. Galls densely aggregated, broadly pyriform, 192–240 μ high by 150–264 μ in broadest diam., oval, 156–170 × 220–245 μ; wall 6 μ thick, hyaline when young, becoming reddish- to dark-brown at maturity; galls becoming cup-shaped when dry.

On *Pectocarya penicilliata*. Leg. C. B. Wolf, Tulare Co., Calif., May 13, 1933; det. C. G. Shaw; Leg. C. W. and H. K. Sharsmith, Stanislaus Co., Calif., Mar. 28, 1935; det. C. G. Shaw. Resting spores up to 3 in a cell, spherical, 120–138 μ, oval, 80–88 × 90–96 μ, with a reddish-brown, smooth, 6 μ thick wall and coarsely granular content. Galls pyriform, 240–288 μ broad by 320–360 μ high with a reddish-brown wall. Several of the resting spores had germinated as a prosorus in these galls and formed a superficial attached sorus of sporangia. The sporangia were predominantly polyhedral, 24–36 μ in greatest diameter.

This is the first report of resting spore germination in *S. myosotidis*, and because the spore functions as a prosorus in the process this species can now be placed with certainty in the subgenus *Pycnochytrium*.

On Plagiobotrys hirtus. Leg. J. R. Anderson, Esquinealt, Vancouver Island, British Columbia, May 10, 1896; det. C. G. Shaw. Resting spores spherical, 110–120 μ, oval, 80–89 × 92–102 μ, or occasionally oblong, 81–90 × 122–132 μ, with a reddish-brown, smooth, 4.6–6 μ thick wall and coarsely granular content; residue lacking. Galls scattered on leaves, dark reddish-brown, hyaline when young, almost spherical, 200–300 μ diam.

SUMMARY

In examining herbarium specimens in various herbaria, the author found several undescribed species of *Synchytrium*. These include *S. polemonii* Ell. & Everh., *S. nitidum* Ell. & Gall., *Synchytrium* spp. on species of *Plantago*, *Sibbaldia* and *Phlox*, and *S. namae* sp. nov., which are described for the first time and included provisionally in the subgenus *Pycnochytrium*. *Synchytrium echii* Speg. was found to be a valid species, but *S. ligusticii* Ell. proved to be a species of *Physoderma* which is probably identical with *P. pluriannulata*. Six new hosts of *S. myosotidis* are recorded, and on *Pectocarya penicilliata* germinated resting spores of the parasite were observed. These had functioned as a prosorus in germination, which shows that *S. myosotidis* is a valid member of the subgenus *Pycnochytrium*.

DEPARTMENT OF BIOLOGICAL SCIENCES,
PURDUE UNIVERSITY,
LAFAYETTE, INDIANA

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TYPE STUDIES OF THE POLYPORES DESCRIBED BY KARSTEN [1]

JOSIAH L. LOWE

In a fifty-year period of intense mycological activity Karsten described 76 species and 22 varieties of polypores. His published works are still the best account of the polypore flora of Finland, and his polypore collections form the bulk of the material from Finland in the Botanical Museum at Helsinki.

The accounts of Karsten's life (**12**; **14**; **25c**, p. 702) are few. His work in polypores is known mostly through his writings, very inadequately and sometimes incorrectly. The type specimens in his own herbarium have never been studied systematically, and collections distributed by him do not always conform to the type material of the same name. He sent a few specimens to Fries, more to Romell and Bresadola and others, and a number were distributed in exsiccati, his own and others. Critical notes on Karsten's species are based for the most part on studies by Bresadola and Romell. These are scattered in the literature and sometimes are in disagreement. In this paper the opinions of the principal students are cited, together with my conclusions based on study of the type material at Helsinki.

The concepts of the species conform to those of Overholts (**30**), and for resupinates, of Lowe (**26**), with references to the current European nomenclature where this differs and is known with certainty by the writer. All of the specimens were studied microscopically.

The work was done in the autumn of 1954. Grateful thanks are due the Museum staff at Helsinki, in particular to Dr. R. Tuomikoski and N. Malmström, who gave every assistance possible. When the material was adequate, permission was given to select a small sample for the National Fungus Collections at Beltsville, Maryland, where it will be more accessible to American students of polypores. Assistance from other individuals is acknowledged under the species concerned.

[1] The work at Helsinki was supported by a research grant from the Mycological Society of America; work at other European herbaria was assisted by a research grant from the National Science Foundation.

ANTRODIA

ANTRODIA SERENA, Soc. F. F. Fenn. Medd. **6**: 10. 1881

The type material is ample and, as well known (**1a**, p. 199, **1d**, p. 254; **2**, p. 231; **3**, p. 667; **8**, p. 69; **11**, p. 144; **24**, p. 20; **31**, p. 420; **34**, p. 21), the species is synonymous with *Poria ancirina* (Sommerf. ex Fries) Cooke.

ANTRODIA SERPENS var. TUBER, Bidrag känn. Finl. Nat. Folk **48**: 324. 1889

The type specimen appears to be a young pulvinate specimen of *Fomes pinicola* (Swartz ex Fries) Cooke.

BJERKANDERA

BJERKANDERA ACRICULA, Rev. Myc. **10**: 73. 1888

The type specimen is *Polyporus immitis* Peck (*P. stipticus* Fries of European mycology). Lloyd (**25b**, p. 375) thought the plant was *Polyporus albellus* Peck from the description; Pilát (**31**, p. 185) quotes Lloyd but adds that perhaps a better disposition would be *Leptoporus stipticus*.

BJERKANDERA ALBOROSEA, Hedwigia **28**: 366. 1889

The type is a poor malformed specimen. The species has been considered a synonym of *Polyporus amorphus* Fries (**2**, p. 250; **9**, p. 166; **31**, p. 153). The external characteristics, septate hyphae, and the few allantoid spores, $5 \times 2\,\mu$, indicate quite clearly that the plant is *Polyporus mollis* Pers. ex Fries.

BJERKANDERA CILIATULA, Soc. F. F. Fenn. Medd. **14**: 80. 1887

The type specimen is very badly damaged by insects. A portion of the upper surface and fragments of the tubes are all that remain. Karsten's illustration (**18b**, no. 54) is scarcely informative. The species has been referred to *Polyporus lacteus* Fries (**2**, p. 192; **3**, p. 539; **31**, p. 185 and 188), and it was unknown to Lloyd (**25b**, p. 377). The type is fertile, and it seems quite certain, in spite of these opinions, that the type specimen is a young strigose example of *Polyporus caesius* Fries.

BJERKANDERA CINERATA, Soc. F. F. Fenn. Medd. **16**: 103. 1889

The type material could be either of two packets with almost exactly the same label. They are the same plant and the better, selected as LECTOTYPE, bears the data, "Surjé ad Abietem, m. Sept. 1889."

The species has been considered a form of *Polyporus fumosus* Pers. ex Fries (*P. imberbis* Bull. ex Fries) by some authors (**2**, p. 242; **3**, p. 551; **31**, p. 164), and it was unknown to Lloyd (**25b**, p. 377). Karsten describes the spores as "longit. 2–3 mmm., crassit. 0.5–1 mmm."; my measurements are 3.5–5 × 1–1.5 μ. The context hyphae are thin-walled, clamped, rarely branched, and mostly 4–5 μ in diameter, varying from 3–6 μ, rather than "2–4 mmm. crassae" as Karsten measures them. The plant is *P. tephroleucus* Fries as Overholts defines it (**30**, p. 297).

BJERKANDERA COLLICULOSA, Hedwigia **29**: 177. 1890.

The type material consists of two packets which are evidently segregates of the same collection. Karsten (**19b**, p. 218) compares his species with *Polyporus trabeus* Rostk. and lists the characteristics by which it differs from that species. The plant is *P. immitis* Peck (*P. stipticus* Pers. ex Fries).

BJERKANDERA IRPICOIDES, Soc. F. F. Fenn. Acta **27(4)**: 5. 1905.

There was no material under this name. Two packets, under separate names, agree with the original description and are presumed to be the original specimens; the LECTOTYPE selected bears the label, "*Bj. fennica*, N. Tugo, Fagervik, 7/X 1896. E. Hisinger." The sporophore is a somewhat unusual form of *Polyporus borealis* Fries.

BJERKANDERA MELINA, Soc. F. F. Fenn. Medd. **14**: 80. 1887

Three packets, all alike and with essentially the same label, could be considered type material. The largest collection with the best material was selected as LECTOTYPE; this could have included the specimen illustrated by Karsten (**18b**, no. 55). The species has been considered a form of *P. lacteus* Fries (**3**, p. 540; **8**, p. 67; **31**, p. 188; **34**, p. 16); Bondarzew (**2**, p. 192) gives it specific rank. The context hyphae are rarely branched, and the specimen agrees with the concept of *Polyporus tephroleucus* Fries of Overholt's manual (**30**, p. 297).

BJERKANDERA MOLLUSCA Rev. Myc. **9**: 9. 1887

The type packet contains several pieces of a small polypore, much discolored and deformed on drying. Lloyd (**25b**, p. 382) thought that this, from the description, appeared to be a white form of *Polyporus amorphus* Fries, and this interpretation has been subsequently followed (**2**, p. 252; **3**, p. 549; **9**, p. 166; **31**, p. 155). The specimens are abundantly fertile and the fungus appears to be the same as *Physisporus aurantiacus* var. *saloisensis* Karsten. See the discussion of that species.

BJERKANDERA PALLESCENS * BJ. PURA, Soc. F. F. Fenn. Medd. **9**: 69. 1882

There are no specimens so named in the Karsten material, but there are four packets which may have been the type material. One, dated October 1860, is selected as LECTOTYPE, although the date does not correspond well to "hieme" in the type description. It is, however, the only collection under the name of *B. pallescens* which could be considered and it is very pale in color. The other packets, variously named, are the same thing. Karsten (**19a**, p. 296) placed this in synonomy with *Polyporus holmiensis* Fries (*Polyporus fumosus* Pers. ex Fries) and Pilát (**31**, p. 161) considers it a doubtful synonym of the same plant. The lectotype specimen is *P. fumosus* (*P. imberbis* Bull. ex Fries of some European mycologists).

BJERKANDERA PELLITA, Hedwigia **34**: 7. 1895

The type material was not found. Karsten, in the original description, notes, "A *Bjerkandera chionea* (Fr.), ad quem proxime accedit, pellicula pilei statim dignoscitur." In a later description (**19d**, p. 75) he gives a wider spore measurement for the species. Lloyd (**25b**, p. 383) and Pilát (**31**, p. 53) consider it a doubtful synonym of *Polyporus osseus* Kalchbr.

BJERKANDERA ROSEOMACULATA, Hedwigia **30**: 247. 1891

The type is a single packet of ample material in good condition. Karsten (**21**, p. 129) later considered it a variety of *Trametes serialis* Fries; Donk (**9**, p. 166) and Pilát (**31**, p. 153) placed it in questionable synonomy with *Polyporus amorphus* Fries.

The type material appears to agree with *Poria monticola* Murr. (*P. microspora* Overh.), and the name is prior. The specimens are better developed than in most American collections. *Physisporus albolilacinus* Karst., is a younger, thinner condition of the same plant.

Dr. Ruth Macrae has kindly compared the type material with *Poria monticola*, and she finds that the sporophore of *B. roseomaculata* appeared chalky and more substantial, the hyphae more often thin-walled and of more irregular wall thickness, and the spores perhaps somewhat more slender than in *P. monticola*. She is uncertain of the synonymy. These variations do not exceed those found in other collections of *P. monticola* that have been confirmed by cultural methods, and the synonymy appears certain.

BJERKANDERA SERPULA, Soc. F. F. Fenn. Medd. **14**: 79. 1887

The type consists of three very small, nearly resupinate sporophores, one growing out over an old specimen of brown polypore. Karsten's illustration (**18c**, No. 66) is not informative. There is no apparent reason for the choice of specific name. Pilát (**31**, p. 617) refers it doubtfully to *Trametes hochnelii* Bres. which it cannot be, as the spores are too wide, 1.5–2 μ, not "crassit, 0.5 mmm." as Karsten states. The plant appears to be a nearly resupinate condition of a species of *Coriolus*, but it is too fragmentary to determine.

BJERKANDERA SIMULANS, Rev. Myc. **10**: 73. 1888

The type collection consists of four small sporophores. Karsten (**19b**, p. 219) states that his plant agrees in its form with *Polyporus radiatus* Sow. ex Fries. Lloyd (**25b**, p. 386) believed that the description pointed to *Polyporus adustus* Sow. ex Fries, an opinion quoted by Baxter (**1k**, p. 21) and Pilát (**31**, p. 157).

The fungus undoubtedly had a white context, and the spores, as Karsten illustrates and states (**18c**, No. 64), are apparently hyaline, allantoid, 4–5 × 1–1.5 μ. The hymenium is badly contaminated. The fungus is not *P. adustus;* certain identification cannot be made, but the fungus appears to agree with *Polyporus tephroleucus* Fries in the sense of Overholts (**30**, p. 297).

The name was changed to *Polyporus karstenii* Saccardo in Syll. Fung. **9**: 170. 1891.

BJERKANDERA SUBSERICELLA, Soc. F. F. Fenn. Medd. **11**: 136. 1884

There are two packets which may be considered type material. The labelling is essentially the same on each and they are the same plant. N. Malmström at the Helsinki Herbarium calls attention to an error in the original description, in which the measurement "2 mm." is erroneously given for 2 cm. The specimens are 2–3 cm broad. The plant was unknown to Lloyd (**25b**, p. 387); Pilát (**31**, p. 176) put it into doubtful synonymy with *Polyporus fragilis* Fries. The specimens are atypical material of *Polyporus floriformis* Quél.

CALOPORUS

CALOPORUS EXPALLESCENS, Soc. F. F. Fenn. Medd. **9**: 110. 1883

The type is a very good specimen. The species was put in synonymy with *Polyporus dichrous* Fries by Romell (**34**, p. 12), but later writers

(**2**, p. 137; **3**, p. 683; **31**, p. 250) recognize it as a distinct species. The type material is *Poria sanguinolenta* (Alb. & Schw. ex Fries) Sacc., differing only in being somewhat thicker than usual, and not discoloring greatly on drying. All intermediates occur with the typical form, and the plant surely does not merit specific rank.

CALOPORUS EXPALLESCENS var. FLAVIDULUS, Soc. F. F. Fenn. Medd. **9**: 110. 1883

The type and only collection is small and on carbonized wood. The species has been placed in synonymy with *Poria reticulata* (Fries) Cooke (**1a**, p. 197; **1f**, p. 303; **31**, p. 429 [doubtfully]; **34**, p. 21). The specimen now shows no trace of the "flavi" color described by Karsten, and it agrees wholly with *P. reticulata*.

CORIOLUS

CORIOLUS APPLANATUS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 3. 1904

The single specimen, the type, is sterile but otherwise is typical *Polyporus pubescens* Schum. ex Fries.

CORIOLUS SIMULANS var. BOREALIS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 3. 1904

Only one specimen was found in the Karsten material to which the description would apply. The variety name is not on the packet; but the description and collection data agree. This specimen is designated LECTOTYPE; it is *Polyporus dichrous* Fries.

CORIOLUS VELUTINUS var. NIGRESCENS, Soc. F. F. Fenn. Acta **27**(4): 5. 1905

The type is old, weathered, and sterile, but is surely *Polyporus hirsutus* Wulf. ex Fries, blackened by exposure, and by dark fungous and algal contaminants.

CORIOLUS VELUTINUS *C. SULCATUS, Trav. Sous-Sect. Troitzk.-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geogr. **8**(1): 61. 1906

The type is a single specimen of *Polyporus hirsutus* Wulf. ex Fries, more deeply sulcate than usual. Bondarzew (**2**, p. 489) considers it a form of *P. hirsutus*.

DAEDALEA

DAEDALEA BETULINA var. POROSA, Sydv. Finl. Polyp. 45. 1859

There are no specimens so named; the variety was probably a somewhat more regularly poroid example of *Lenzites betulina* (L. ex Fries) Fries.

DAEDALEA CANDICANS, Trav. Sous-Sect. Troïtzk.-Kiakhata, Sect. pays d'Amour Soc. Imp. Russe Geogr. **12**(1 & 2): 110. 1911

The single type specimen is fertile, and it is a form of *Daedalea confragosa* Bolt. ex Fries with pale upper surface, poroid below, with tubes split to produce a distinctly hydnoid appearance.

DAEDALEA OUDEMANSII var. FENNICA, Soc. F. F. Fenn. Medd. **9**: 69. 1882

The type collection has been badly damaged by insects. The context tissue has been mostly destroyed and very little of the surface covering remains; the tubes, however, are intact and the specimen is fertile, with spores oblong-ellipsoid, 4–5 × 2.5 μ. The fungus is a young condition of *Polyporus adustus* Willd. ex Fries.

DAEDALEA VARIEGATA, Trav. Sous-Sect. Troïtzk.-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geog. **12**(1 & 2): 111. 1911

The type specimen is a form of *D. confragosa* Bolt. ex Fries, with very fine, faint zonations. The type packet data differs from the type description in the date, which is 1909 on the packet (instead of 1908), and the substratum, "ad pinum" on the packet. The type description phrase "In trunco Salicis" was evidently taken from another packet, a collection by Bondarzew from Siberia.

DAEDALEOPSIS

DAEDALEOPSIS INCANA, Finska Vet.-Soc. Öfv. Förh. **46**(11): 4. 1904

The type material is three very poorly developed, obviously immature sporophores with hymenial surface just forming. The material is fertile, and the fungus appears to be *Daedalea unicolor* Bull. ex Fries, as Bondarzew (**2**, p. 478) also refers it. Karsten (**23**, p. 110) considered it very similar to *Antrodia serpens* (Fries) Karst.

FOMES

FOMES ABIETIS, Bidrag känn. Finl. Nat. Folk **37**: 242. 1882

Only one specimen agrees with the type locality data and this, from Jacobstad, is designated the LECTOTYPE. It is the annual form of *Fomes pini* (Thore ex Fries) Karst. on spruce, as are a number of other collections so determined by Karsten. All are annual with the exception of one which had renewed growth after the position of the sporophore had changed. It is not evident from the material why Karsten placed the species in the genus *Fomes*. The species has been considered valid (**3**, p. 633; **4**, p. 89; **9**, p. 252; **25b**, p. 277; **34**, p. 26), as a variety of *F. pini* (**1b**, p. 427; **2**, p. 384; **30**, p. 79; **31**, p. 520), and as a synonym of *F. pini* (**28**, p. 111), to which position the writer would also assign it.

FOMES CAJANDERI, Finska Vet.-Soc. Öfv. Förh. **46**(11): 8. 1904

The type material is in two packets, evidently segregates of the same collection. The species was unknown to Lloyd (**25b**, p. 278). The specimens are fertile and are the same as *Trametes subrosea* Weir, a determination kindly confirmed by Dr. Ruth Macrae. Karsten's name has priority, as Weir's name was published in 1923.

Pycnoporus mimicus is the same plant.

FOMES ROBUSTUS, Bidrag känn. Finl. Nat. Folk **48**: 467. 1889

This species was based upon a single sporophore which was first noted by Karsten (**17**, p. 74) as *"Ischn.? dryadeum"* (Pers. ex Fries) Karst. and later determined to be a distinct species. The sporophore was broken and divided between four packets, only one under the proper name. The sporophore is an old and weathered one, with a few spores along the hymenium and with rare ventricose setae about 16–19 × 7–9 μ. The species is certainly correctly interpreted by all later writers (**1b**, p. 424 and **11**, p. 103; **2**, p. 361; **3**, p. 616; **6**, p. 38; **25b**, p. 242; **27**, No. *245*; **30**, p. *87*; **31**, p. 504).

FOMES TENUIS, in Roumeguère, Fungi Gallici Exs., No. *4019*. 1887

There is no exsiccati specimen in the Karsten Herbarium. Those in other herbaria, and in addition two resupinate and one pileate specimen so named by Karsten are the plant widely known under this name and the same as Fries' identified material of *Polyporus isabellinus* Fries at Upsala, as many others have stated (**1b**, p. 427 and **1e**, p. 292; **2**, p. 388; **3**, p. 622; **8**, p. 68; **11**, p. 167; **24**, p. 19; **27**, No. *247*; **31**, p. 542; **34**, p. 24). Lloyd (**25b**, p. 285) thought the plant was *Poria ferrea* (Pers.) Bourd. & Galz.

FOMES THELEPHOROIDES, Rev. Myc. **9**: 9. 1887

There are two packets, evidently from the same collection, which probably served for Karsten's illustration (**18b**, No. 57). The species was unknown to Lloyd (**25b**, p. 286); Pilát (**31**, p. 621) is uncertain of its identity; and Laurila (**24**, p. 19) refers it to *Trametes trabea* (Pers. ex Fries) sense of Bourdot & Galzin (**3**, p. 586; = *T. protracta* Fries). The specimen is quite surely referable to *T. protracta*, a determination kindly confirmed by Dr. S. Lundell.

HANSENIA

HANSENIA IMITATA, Soc. F. F. Fenn. Medd. **13**: 161. 1886

The type specimen was not found. Karsten (**19**, p. 304) placed his species in synonymy with *Polyporus pubescens* Schum. ex Fries, and Pilát (**31**, p. 603) agrees.

INONOTUS

INONOTUS LEVIS, Hedwigia **26**: 112. 1887

The type collection is a single sporophore, as illustrated (**32**). Karsten (**19d**, p. 82) placed his species in synonymy with *Inonotus fulvus* (Fries) Karst. The specimen is excellent, and agrees exactly with *Polyporus dryophilus* Berk. (*P. rhcades* Pers.; *P. vulpinus* Fries) except that the spores are somewhat larger than in the typical form, 6.5–9 × 5–6 μ. This size agrees rather well with the measurements given by Bourdot and Galzin (**3**, p. 637) for *Xanthochrous tamaricis* Pat., but separate names for forms in this highly variable species are of extremely doubtful usefulness.

INONOTUS RADIATUS var. SCROBICULATUS, Bidrag känn. Finl. Nat. Folk **37**: 238. 1882

Only one specimen, "Mustiala, ad Syringam, m. Aprili 1882" could be found that corresponds with the original description, and this is selected as LECTOTYPE. Bondarzew (**2**, p. 324) and Pilát (**31**, p. 556, doubtfully) place the species in synonymy with *Polyporus radiatus* Sow. ex Fries. Lloyd (**25b**, p. 385) says, "Description reads like radiatus. Spores read like cuticularis. Unknown what it is." The lectotype, and another specimen on *Syringa*, Mustiala 29 March 1882, are *P. radiatus*.

INONOTUS SULPHUREOPULVERULENTUS, Finska Vet.-Soc. Öfv. Förh. **46**(11) : 8. 1904

The type, a fragment of a sporophore, is *Polyporus schweinitzii* Fries. Pilát (**31**, p. 620) considers it a species of uncertain identity.

INONOTUS TRIQUETER var. PURPURASCENS, Bidrag känn. Finl. Nat. Folk **48** : 331. 1889

The only collection, the type, is a small specimen with the hymenium in poor condition, but surely referable to *Polyporus mollis* (Pers. ex Fries) Fries. Pilát (**31**, p. 583) puts it in questionable synonymy with *P. tomentosus* Fries.

INONOTUS UFENSIS, Finska Vet.-Soc. Öfv. Förh. **46**(11) : 3. 1904

There are two packets in the Karsten material, segregates apparently of the same collection. The fungus is a labyrinthiform condition of *Trametes protracta* Fries.

INONOTUS VULPINUS var. HISINGERI, Bidrag känn. Finl. Nat. Folk **37** : 238. 1882

The type was not found. Saccardo (Syll. Fung. **6** : 119) changed the name to *Polyporus inonotus* Karsten without explanation, and this name was also used by Bresadola (**6**, p. 38).

LENZITES

LENZITES AMBIGUA, Soc. F. F. Fenn. Acta **27**(4) : 5. 1905

The type material is adequate and fertile, and appears to be a dwarf form of *Polyporus abietinus* Dicks. ex Fries, as Pilát (**31**, p. 591) has also referred it.

LENZITES LARICINA, Soc. F. F. Fenn. Acta **27**(4) : 4. 1905

The type collection is a small form of *Lenzites abietina* Bull. ex Fries with woolly upper surface. Pilát (**31**, p. 274 and 276) refers it doubtfully to a form of *Polyporus abietinus* Dicks. ex Fries, and Bondarzew (**2**, p. 566) refers it doubtfully in synonymy with *Xylodon candidum* f. *lenzitoideum* (Murash.) Bond.

LENZITES PINICOLA, Trav. Sous-Sect. Troïtzk.-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geog. **8**(1) : 62. 1906

There are two packets, both apparently from the same collection. The fungus is a tomentose, sulcate form of *Lenzites abietina* Bull. ex Fries, but with spores somewhat smaller than usual, 7–9 × 2.5–3 μ.

LENZITES SEPTENTRIONALIS, Bidrag känn. Finl. Nat. Folk **25**: 239. 1876

Two of the collections enumerated in the description are now at Helsinki; the collection by Karsten at "Soukelo ad Betulam, 6 Aug. 1861" is considered the lectotype. The identity of the fungus was uncertain to Pilát (**31**, p. 617). The fungus is a lamellate form of *Daedalea confragosa* Bolt. ex Fries. Another collection determined by Karsten from Knäsä is the same.

The publication cited by Karsten in the 1876 description was apparently not published until 1882 (in Soc. F. F. Fenn. Not. Sällsk. Förh. **8**: 199).

LENZITES SIBIRICUS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 3. 1904

The three specimens in the type collection are poorly developed, reddish forms of *Daedalea confragosa* Bolt. ex Fries. Bondarzew (2, p. 574) considers it a form of *D. confragosa*.

LENZITES SIBIRICA var. UNIZONATA, Trav. Sous-Sect. Troïtzk.-Kiakhta, Sect. pay d'Amour, Soc. Imp. Russe Geog. **12**(1 & 2): 111. 1911

There is only one collection in the Karsten material which can be considered the type. It was labelled "*Lenzites tricolor*." It agrees with the original description except that it is scarcely "concentrice sulcata." The upper surface has faint, narrow zonations in the outer 2 centimeters, within roughened and the zonations lacking. The under surface has somewhat anastomosing lamellae. The name is of poor application.

The fungus is typical of *Daedalea confragosa* Bolt. ex Fries.

LENZITES SORBINUS, Bidrag känn. Finl. Nat. Folk **37**: 54. 1882

The type is in fragmentary condition, nearly destroyed by insects. The upper surface was evidently hirsute and the pores more or less daedaloid and about 1–2 per mm. The fungus is probably *Lenzites betulina* (L. ex Fries) Fries.

PHYLLODONTIA

PHYLLODONTIA MAGNUSII, Hedwigia **22**: 163. 1883

The type was not found. Dr. R. Tuomikoski reported that the only specimen in the Karsten Herbarium was No. *3738* of Rabenhorst-Winter, Fungi Europaei. That specimen is sterile, with numerous cystidia of highly variable shape. It may be a variety of *Daedalea unicolor* Bull. ex Fries, as has been stated (**2**, p. 478; **3**, p. 564; **31**, p. 280).

PHYSISPORUS

PHYSISPORUS ALBOATER, Hedwigia **31**: 293. 1892

The type of this is a form of *Poria sanguinolenta* (Alb. & Schw. ex Fries) Cooke that blackened at the pore surface when it dried.

Another collection so determined by Karsten, but from another locality, is wholly different. It is sterile and its identity is unknown.

PHYSISPORUS ALBOLILACINUS, Hedwigia **31**: 220. 1892

The type is a younger and thinner condition of *Bjerkandera roseomaculata*. Dr. Ruth Macrae has kindly compared the type material with *Poria monticola*. As compared with that species, the sporophore of *P. albolilacinus* is very similar in macroscopic appearance, but the majority of the hyphae are thin-walled, with only a few thick-walled and with evenly thickened walls, in contrast with *P. monticola*, in which most of the hyphae are thick-walled with the walls fairly regular in thickness. She is uncertain of the synonymy. These differences appear to be within the permissible range of variation within the species, and it is considered the same as *Poria monticola*

PHYSISPORUS AURANTIACUS var. SALOISENSIS, Rev. Myc. **9**: 10. 1887

The type material is adequate and fertile. Pores about 5 per mm; spores short-cylindrical to narrowly ellipsoid, 4–5 × 1.5–2 μ. The plant has been considered the same as the species by several workers (**2**, p. 269; **3**, p. 665; **5**, p. 77 [as *P. nitida* (Pers.)]; **31**, p. 398). The type agrees with the usual concept of *P. aurantiaca* except as to size of pores, which are "minuti" as Karsten describes them. This difference is scarcely enough to separate a variety, particularly in a species that changes so markedly on drying.

It should be noted that the varietal name was misspelled *"taloisensis"* in the original description.

PHYSISPORUS CAESIO-ALBUS, Hedwigia **22**: 177. 1883

The type material is the pale resupinate form of *Polyporus abietinus* Dicks. ex Fries, as many others have stated (**1j**, p. 194; **2**, p. 561; **3**, p. 567; **4**, p. 87; **31**, p. 273 and 275).

PHYSISPORUS CRASSUS, Bidrag känn. Finl. Nat. Folk **48**: 319. 1889

There are 2 collections in the Karsten material that could be considered type. They are the same plant apparently. A small portion of a sporophore, labelled June 6, 1872, is fertile, in better condition, and is

considered the LECTOTYPE; a September 1886 collection is much more ample, but sterile.

This species has been considered a synonym of *Poria xantha* (Fries) Cooke by Egeland (**11**, p. 150) and Baxter (**1d**, p. 255, as a form). The first intimation that it might be a valid species was by Romell (**36**, p. 22) who said that it may "perhaps be a distinct species," an opinion probably based on a Karsten specimen in his own herbarium (now at the Riksmuseet) that is annotated by him "spores ellipt. 4–5 × 2½–3 μ." Bourdot and Galzin (**3**, p. 691) credit Romell with the observation that the spores are ellipsoid and recognize it as a distinct species, as do others (**1a**, p. 201; **2**, p. 313; **13**, p. 22 [in the best account available]; **26**, p. 73; **29**, p. 26; **31**, p. 463). It is a valid species.

The sporophore resembles externally *Poria xantha* and *P. oleagina* Overh., but differs in the microscopic structure, particularly the hyphae and the oblong-ellipsoid spores.

PHYSISPORUS (ANTRODIA) EUPATORII, Rev. Myc. **6**: 214. 1884

There was no specimen filed under this name in the Karsten material, but there was a packet under a herbarium name that agrees in every detail except minor variations in the locality data. Within the packet is an envelope addressed to the collector, Letendre, apparently the envelope in which the specimen was enclosed when received by Karsten.

The material is exceedingly scanty. There are 4 tiny pieces of herbaceous stem, with a very thin and inconspicuous fungous growth.

The fruiting body closely resembles *Poria fatiscens* (Berk. & Rav.) Cooke externally, but the regularly clamped hyphae and wider spores seem adequately to differentiate it. According to the description by Burt (Mo. Bot. Gard. Ann. **4**: 340. 1917) and material determined by Romell it would appear to be very similar to *Merulius serpens* (Tode ex Fries) Fries sense of Romell, differing chiefly in having a fugitive color reaction with KOH solution. As its identity is uncertain, a description is supplied.

Pore surface pale pinkish-brown, the tubes less than 1 mm long; pores at first merulioid, mere depressions in the context, later well-defined and 2–3 per mm, the dissepiments remaining obtuse and rather thick; context and tubes at first pink then decolorized in KOH, the hyphae clamped, 3–4 μ in diameter; basidia about 13 × 5 μ; cystidia none; spores hyaline, smooth, oblong-ellipsoid to ellipsoid, 4–5 × 2–2.5 μ.

PHYSISPORUS FLAVICANS, Hedwigia **35**: 44. 1896

There is only one collection in the Karsten material which can be considered type material. This is a rather poorly developed sample

which had evidently grown on a nearly vertical substrate, and the tubes are consequently poorly developed. Two kinds of spores were found along the hymenium, one cylindrical, 4–5 × 1.5 μ, the other allantoid, about 3–4 × 0.5 μ. The latter is considered to be a contaminant. There are very large incrusted cystidia in the hymenium. The fungus is certainly a synonym of *Physisporus luteoalbus* Karst., described in 1887.

The original description says "cystidia nulla." Karsten may not have seen the cystidia on the type specimen, or, more likely, he may have examined for this character the one other collection (Mustiala, 23 October 1866) which is included in a later description (**19d**, p. 79), where he compared his plant to *Poria mollusca* (Fries) Cooke. This second plant is the yellow *Poria* which Romell (**34**, p. 25; **36**, p. 11) took for the species, a concept which has been followed by Egeland (**10c**, p. 380 and **11**, p. 148); and Baxter (**1a**, p. 214 and **1d**, p. 253).

No name is known for the yellow *Poria* which has passed under the name of *Poria flavicans* Karst., but such a striking plant has certainly been described elsewhere. A search should be made for it.

PHYSISPORUS FRAGILLIMUS, Bidrag känn. Finl. Nat. Folk **62**: 79. 1903

There are 8 collections in the Karsten material which could have formed the basis for the description, *i.e.*, collected Aug.–Nov. 1898 and earlier. Five of these are referable to *Poria candidissima* (Schw.) Cooke, one to *P. sericeomollis* (Rom.) Baxter, one to the yellow *Poria* referred to *P. flavicans* Karst. by Romell, and one somewhat similar to the last, but with different spores, a species unknown to me.

The original description states that the spores are globose or elliptical-globose, 3 × 2 or 2 μ in diameter. The specimens that are *P. candidissima* are the only ones which agree with this description. The LECTOTYPE selected is annotated, "Mustiala in ligno pini, P. A. Karsten, 8 Sept. 1870." The species is considered a synonym of *P. candidissima*. Baxter (**1a**, p. 196) and Pilát (**31**, p. 389, "teste Romell") give the same disposition.

PHYSISPORUS INCONSTANS, Rev. Myc. **9**: 10. 1887

The type collection is ample and in good condition. The species is a synonym of *Poria viridans* (Berk. & Br.) Cooke, as Karsten himself considered possible (**19d**, p. 80, citing Bresadola's opinion), and as Bresadola (**4**, p. 83, **8**, p. 69) pointed out and later writers concurred with (**1a**, p. 202; **1g**, p. 177; **2**, pp. 140–141; **3**, p. 661; **9**, p. 171; **11**, p. 154; **31**, p. 402; **35**, p. 644).

PHYSISPORUS LACER, Soc. F. F. Fenn. Medd. **9**: 69. 1882

The three specimens determined by Karsten are all alike. The plant is allied to *Polyporus tulipiferae* (Schw.) Overh., but differs in having smaller spores and clamped hyphae. It may have been described in the genus *Irpex*. It was unknown to Pilát (**31**, p. 605), and is unknown to me.

PHYSISPORUS LENIS, Rabenhorst-Winter, Fungi Eur. et Extraeur.: Cent. **16** (or **36**), No. *3527*. 1886

The LECTOTYPE selected for this species is the specimen in the bound Rabenhorst at Helsinki, which is an unmixed collection and fertile, with the lunate spores unique for this species. All of the several packets studied of No. *3527* contain the species exemplified by the lectotype, and two of them, the unbound packet at Helsinki and the packet in the Riksmuseet, contain also a small sample of *Poria sericeomollis* (Romell) Baxter. The species has been correctly known under its own name (**1d**, p. 248; **2**, p. 149; **4**, p. 87; **24**, p. 20; **31**, p. 441; **34**, p. 17; **36**, p. 12), or as a variety of *Poria calcea* (**3**, p. 674), or closely related to it (**11**, p. 149). Eriksson's excellent description (**13**, p. 11), which includes allantoid spores for the species, constitutes an emended concept.

PHYSISPORUS LOCELLATUS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 8. 1904

There are 2 packets which may be considered SYNTYPES. The plants are myriadoporus; hyphae nonseptate, 3–5 μ in diameter; spores hyaline, smooth, cylindrical, apiculate, 8–11 × 3–3.5 μ. The relationships of the plant are unknown to me.

PHYSISPORUS LUTEOALBUS, Rev. Myc. **9**: 10. 1887

There are 8 collections in the Karsten material which may have been studied for the original description. Three of these, collected in 1886, are the only ones that wholly fit the wording of the original description. The collection of "13 Oct., 1886, Mustiala, in ligno pini," is selected as the LECTOTYPE. These three agree with the concept of Eriksson (**13**, p. 15). The other collections are referred thus: two to *Poria lenis* Karst.; one, sterile and resupinate, probably to *Polyporus semisupinus* Berk. & Curt.; one to *Polyporus sericeomollis* Romell, and one poor and unknown to me.

Bresadola (**4**, p. 86) considered this a form of *Poria vulgaris* (Fries). This concept was rejected by Karsten (**19d**, p. 81), but was followed by Egeland (**11**, p. 138) and Bourdot and Galzin (**3**, p. 675

[as a variety of *P. calcea*]), and Starbäck (**37**). Baxter variously refers it to *P. biguttulata* Romell (**1a**, p. 202), to *P. xantha* (**1d**, p. 257) and to *P. luteoalba* in the sense of Eriksson (**1m**, p. 136), and Pilát (**31**, p. 444) considers it a doubtful synonym of *P. xantha* (Fries) Cooke, "teste Romell" (published?). Bondarzew (**2**, p. 174) refers to Eriksson's description and evidently accepts it. Lundell and Nannfeldt (**27**, Nos. *1826, 1827*) accept it.

Karsten's description of the spores "oblongatae vel subellipsoideae, rectae; longit. 3–6 mmm., crassit. circiter 2 mmm." fits only the material with large cystidia, and Eriksson has surely correctly interpreted the species.

The species is apparently antedated by *Physisporus variecolor*, which see.

PHYSISPORUS MAKRAULOS var. NIGROMARGINATUS, Bidrag känn. Finl. Nat. Folk **37**: 58. 1882

The type specimen was not found. According to Pilát (**31**, p. 362 and 364) this name was used for forms of the plant now known as *Fomes annosus* (Fries) Cooke.

PHYSISPORUS MICROSPORUS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 8. 1904

The type, only specimen in the Karsten material, is sterile, but otherwise agrees wholly with *Poria nigrescens* Bres. and is considered a synonym of it. Bondarzew (**2**, p. 51) recognizes the species.

It should be noted that the published name is a typographical error. The original packet spells the specific name "*micropora*" and the original description states that the pores are "minutissimi," but gives no information on the spores.

PHYSISPORUS MOLLUSCUS *BOMBYCINOIDES, Soc. F. F. Fenn. Medd. **11**: 21. 1884

The two packets in the Karsten material, presumably both part of the type collection, agree with *Poria vaillantii* (DC. ex Fries) Cooke. The specimens are sterile, but otherwise typical. Pilát (**31**, p. 593) apparently considers this a variety of *Poria mollusca* (Pers. ex Fries) Cooke.

PHYSISPORUS NITIDUS *PH. VITELLINULUS, Bidrag känn. Finl. Nat. Folk **37**: 59. 1882

Apparently a change of name for *P. vitellinus*, which see.

PHYSISPORUS TENER, Rev. Myc. **12**: 128. 1890

The type specimen is sterile, but otherwise agrees perfectly with *Poria corticola* (Fries) Cooke. The species has been placed in synonymy with *Poria cupora* (Karst.) Cooke (**9**, p. 217 [spelled "*europaca*"]; **34**, p. 12). This species was made the type of a new genus *Chaetoporus* by Karsten (**20a**, p. 148) the same year.

PHYSISPORUS (ANTRODIA) TUBER, Hedwigia **24**: 72. 1885

The type is a deformed poroid specimen of some species of *Daedalea*, *Lenzites*, or possibly *Trametes*, distinctive because the tissue turns quite black in KOH.

PHYSISPORUS VARIECOLOR, in Thuemen, Mycotheca Univ., Cent. 19, No. *1803*. 1881

Material distributed in the exsiccati set is of varied identity. Five (at Paris, Upsala [the only fertile specimen], Farlow Herbarium, N. Y. Bot. Garden [Ellis collection], and Beltsville) agree with *Poria luteoalba* (Karst.) Sacc.; at Helsinki the specimen is *Poria candidissima* (Schw.) Cooke; and at Kew it is surely *Poria lenis* (Karst.) Sacc. Additional material so labelled by Karsten at Helsinki and New York are *Poria subacida* (Peck) Sacc.

As should be expected the species has had varied treatment. It usually has been considered a synonym of *Poria unita* (Pers.) Cooke (**1h**, p. 154; **31**, p. 466) or of its variant *Poria pulchella* (Schw.) Cooke (**2**, p. 312; **3**, p. 684; **8**, p. 69; **10c**, p. 383; **11**, p. 150), or a synonym of *Poria xantha* (Fries) Cooke (**31**, p. 444).

All or nearly all of these opinions quote Bresadola's work, which probably had as its basis the specimen in the Bresadola Herbarium cited by Baxter (**1h**, p. 154). This specimen was not found in the Bresadola Herbarium and, if correctly cited, is not type material, as it was on *Alnus incana*, not *A. glutinosa* as in the original description.

The predominant material in the Karsten exsiccati is *Poria luteoalba*. These specimens agree with the original description, and appear to be the plant to which Karsten's name should apply. If correctly interpreted *P. variecolor* is the prior and valid name.

PHYSISPORUS VITELLINUS, Soc. F. F. Fenn. Medd. **6**: 5. 1881

The type is immature material of *Poria pulchella* (Schw.) Cooke. Baxter (**1d**, p. 257) places the species in synonymy with *Poria xantha* (Fries) Cooke.

Physisporus nitidus **Ph. vitellinulus* is represented by a single packet with exactly the same label except for name as the type of *P. vitellinus*. It is the same plant and appears to have been a part of the same collection. Apparently the fungus was renamed, without explanation or reference to the previous name.

The variety was accepted as a valid species by Egeland (**11**, p. 150), as *Poria vitellinula*. Mostly, however, it has been considered a member of the *Poria unita* complex, as *P. unita* (Pers. ex Fries) Cooke (**1h**, p. 154), *P. medulla-panis* (**31**, p. 466), as *P. tenuis* (**2**, p. 312), and as *P. pulchella* (**3**, p. 684; **8**, p. 69).

The type packet contains immature *Poria pulchella*.

PODOPORIA

PODOPORIA CONFLUENS, Hedwigia **31**: 297. 1892

There are two packets with the same data in the Karsten material. They are poor sterile specimens. The species has been placed in synonymy with *Poria sanguinolenta* (Alb. & Schw. ex Fries) Cooke (**9**, p. 158; **31**, p. 248) but the context hyphae are wholly different than in that species. Spores must be found to determine the species with certainty, but otherwise it agrees well with *Poria pannocincta* (Romell) Lowe. If the synonymy is established Karsten's name would be prior.

POLYPILUS

POLYPILUS CONGLOBATUS, Finska Vet.-Soc. Öfv. Förh. **46**(11): 2. 1904

No specimen agreeing with the description was found under this name, but a specimen under a herbarium name otherwise agrees completely and is considered the type. The species has been referred doubtfully to synonymy with *Polyporus biennis* var. *distortus* Schw. (**25a**, p. 161; **31**, p. 596), to which it probably does not belong, as there are no chlamydospores and no cystidia.

The plant is a malformed specimen, with a very poor development of tubes. It is unknown to me. There are two specimens collected later and so named by Karsten and they too are indeterminable by me.

POLYPORELLUS

POLYPORELLUS ALBULUS, Hedwigia **33**: 15. 1894

The single specimen of the type appears to be a pale centrally stipitate form of *Polyporus floriformis* Quél.

POLYPORELLUS CAUDICINUS *P. SORORIUS, Trav. Sous-Sect. Troïtzk.-Kiahkta, Sect. pays d'Amour Soc. Imp. Russe Geog. **8**(1) : 61. 1906

The type specimen was not found. According to the description it differs from typical *Polyporus sulphureus* Bull. ex Fries by polished spots on the pileus and a unicolorous stipe.

POLYPORELLUS LEPIDEUS var. SUBMURINUS, Soc. F. F. Fenn. Acta **11**(1) : 30, 1881 (*nomen nudem*) ; Bidrag känn. Finl. Nat. Folk **37** : 28. 1882

The type was not found. The variety is put into doubtful synonymy with *P. brumalis* f. *trachypus* Rostk. by Pilát (**31**, p. 69). Bresadola (**7**, p. 225) says that Karsten's specimens of *P. lepideus* are juvenile forms of *P. brumalis* and the specimens so named in his herbarium are *P. brumalis*.

POLYPORELLUS LEPTOCEPHALUS var. TERRIGENUS, Bidrag känn. Finl. Nat. Folk **37** : 30. 1882

The type specimen was not found. According to Pilát (**31**, p. 108) the specific name was used for plants now considered forms of *Polyporus varius*, and specimens in his herbarium so named by Karsten are in that group of related species.

POLYPORELLUS MELANOPUS *P. HISINGERI, Hedwigia **35** : 173. 1896

The type is a fine specimen of the same or very similar plant which has been called *Polyporus fagicola* Murr. in America, differing in being a much larger specimen. The relations of the subspecies is with the *Polyporus squamosus* Huds. ex Fries group, not with the *P. melanopus* Swartz ex Fries group.

POLYPORELLUS VARIUS (Fr.) *P. TUBAEFORMIS, Soc. F. F. Fenn. Medd. **9** : 69. 1882

The type material consists of 2 sporophores, probably immature, and these appear to be a thin umbilicate form of *Polyporus varius* Fries, as Lloyd (**25a**, p. 188) placed it. Karsten's illustration (**18b**, No. 53) is not decisive. Pilát (**31**, p. 103) and Bondarzew (**2**, p. 457) recognize it as a form of *Polyporus picipes* Fries.

POLYPORUS

POLYPORUS AMORPHUS var. ALBUS, Sydv. Finl. Polyp. **29**. 1859

The specimen selected as LECTOTYPE is annotated, "Ad truncos abiegnos prope Helsingfors, W. Nylander Oct. 1858." It is badly

damaged by insects but is fertile and is referred with certainty to *Trametes serialis* Fries. It is entirely dissimilar to *P. amorphus* Fries in consistency and internal structure.

Another packet annotated "N. Helsingfors, på Pinus stubbar 1858, Herb. W. Nylander" is the same, but a third packet, with exactly the same label as the latter specimen, is sterile and appears to be *Trametes hispida* Bagl. (surely a repacketing error).

POLYPORUS CONTIGUUS *P. RIXOSUS, Bidrag känn. Finl. Nat. Folk **25**: 272. 1876

There are 7 specimens which may have been considered in making the original description. Five of these are apparently alike and the only fertile one is selected as LECTOTYPE, collected at Wasa 12 Aug. 1867. Two lack cystidia and by texture appear to be a different plant. Since sterile, no determination was made.

The concept is the one current in Europe and America for this species (**1a**, p. 211; **2**, p. 173; **3**, p. 676; **11**, p. 158; **26**, p. 36; **29**, p. 50; **31**, p. 447; **34**, p. 13).

It should be noted that *Polyporus collabens* Fries is a prior name, according to the Friesian material at Upsala, which agrees in every detail except it is sterile. I am indebted to Dr. Seth Lundell for consideration of this species.

POLYPORUS EUPORUS, Fungi Fenniae Exs., Cent. VII, No. *618*. 1867

The exsiccati specimen at Upsala is selected as the LECTOTYPE. Century VII is lacking at Helsinki; 2 collections dated 21 October 1866 are the same plant and all agree with the long-accepted concept (**1a**, p. 210; **2**, p. 170; **3**, p. 678; **26**, p. 26; **27**, No. 67; **29**, p. 45; **31**, p. 455; **34**, p. 12). It is said to agree with *Poria blytii* (Fries) Sacc. in part (**4**, p. 82; **36**, p. 10), or to be a variety of it (**5**, p. 78), or a synonym of *Poria nitida* (**11**, p. 151), and Romell believes (**36**, p. 10) it is "probably identical with *Pol. rhodellus* Fr. and *Poria nitida* Pers. and . . . *Poria micans* Ehr." It seems best to use Karsten's name for this plant, as it is certain.

POLYPORUS FIBRILLOSUS, Sydv. Finl. Polyp. 30. 1859

The type material consists of one-half of a small sporophore, and it is a valid species as has long been accepted (**1m**, p. 134; **2**, p. 265; **3**, p. 558; **28**, p. 70; **30**, p. 381; **31**, p. 142). Lloyd (**25b**, p. 378) thought Karsten was confused as to the identity of this species, but Karsten's material is consistent.

POLYPORUS FLAVUS, Sydv. Finl. Polyp. 40. 1859

The type is an excellent fertile collection and is *Poria xantha* (Fries) Cooke, as has been stated (**1a**, p. 202; **1d**, p. 257; **31**, p. 444 [doubtfully]). Karsten (**15**, p. 276; **17**, p. 62) later considered it a variety of *Polyporus vulgaris* Fries, as did Romell (**34**, p. 25).

The name was changed to *Polyporus selectus* by Karsten in 1868.

POLYPORUS HIANS, Fungi Fenniae Exs., Cent. VII, No. *619*. 1867

The LECTOTYPE selected for this species is the specimen in the exsiccati set at Upsala. This Century is lacking at Helsinki; 5 collections so named by Karsten agree with the lectotype specimen. The plant is a synonym of *Poria bombycina* (Fries) Cooke, as has long been known (**1j**, p. 204; **2**, p. 124, 125; **3**, p. 670; **4**, p. 81; **8**, p. 69; **10b**, p. 90; **11**, p. 152; **24**, p. 20; **31**, p. 431).

POLYPORUS PALLESCENS "Karst.," in Romell, Arkiv. f. Bot. **11**(3): 19. 1912

There are no specimens in the Karsten material which indicate that he recognized the plant as a separate species. There are specimens under this name attributed to Fries and in print Karsten repeatedly attributed the species to Fries. Romell attributed the species to Karsten apparently because Karsten had sent him a misnamed specimen growing on the hymenium of *Polyporus fomentarius* L. ex Fries. Frequently recognized as a valid species (**1a**, p. 215; **1i**, p. 217; **3**, p. 569; **10c**, p. 379); it is on the other hand considered a synonym of *Polyporus semisupinus* Berk. & Curt. (**2**, p. 162; **27**, No. *2104*; **31**, p. 212). Most of Romell's type material is now at the Riksmuseet in Stockholm; these are the resupinate condition of *P. semisupinus*.

POLYPORUS ROSTAFINSKII, Bidrag känn. Finl. Nat. Folk **25**: 274. 1876

The two packets agreeing with the original data are evidently parts of the same collection, and the better packet was selected as LECTOTYPE. The material is sterile, but certainly the same as *Poria corticola* (Fries) Cooke, as the species has been referred by Karsten himself (**19c**, p. 178; **20c**, p. 293) and others (**2**, p. 178; **3**, p. 692; **31**, p. 448 [doubtfully]; **36**, p. 15). It was unknown to Bresadola (**4**, p. 87).

POLYPORUS SELECTUS, Soc. F. F. Fenn. Not. Sällsk. Förh. **9**: 360. 1868

This is a new name for *Polyporus flavus* with, however, a different type specimen. The type is *Poria xantha* (Fries) Cooke, as has been long recognized (**1a**, p. 202; **1d**, p. 257; **2**, p. 153; **11**, p. 150; **31**, p. 444 [doubtfully]; **34**, p. 25).

POLYPORUS SOMMERFELTII, Soc. F. F. Fenn. Medd. **2**: 181. 1878

A *nomen nudum*. The species was placed in synonymy with *Trametes mollis* Sommerf. by Karsten (**17**, p. 53).

PORIA

PORIA CANESCENS, Rev. Myc. **9**: 10. 1887

Two collections, the same plant, could be considered type material. The lectotype selected by Rogers bears the data "Mustiala, Sarkjarvi ad Alnum, 7 XI 1886 leg. PAK." Long recognized as a species in the Polyporaceae (**1h**, p. 161; **2**, p. 158; **3**, p. 689; **29**, p. 24; **31**, p. 418), it has been found recently by Teixeira and Rogers (**38**) to have cruciately divided basidia. Cruciate basidia were clearly seen on the lectotype material. The valid name is *Aporpium caryae* (Schw.) Teixeira & Rogers and the family is the Tremellaceae.

PORIA CARBONICOLA, Finska Vet.-Soc. Öfv. Förh. **46**(11): 4. 1904

The type collection, and another one by the same collector from the same place, are sterile, and the hymenium is infested with a hyphomycete. The identity of the species will be uncertain until spores are found.

PORIA FERRUGINEOFUSCA, Soc. F. F. Fenn. Medd. **14**: 82. 1887

Of the 4 packets that agree in part with the type description, the unbound packet from Rabenhorst's Fungi Europaea No. *3645* at Helsinki agrees best and is selected as LECTOTYPE. All of the material so named by Karsten is the same species, and agrees with the current concept of a brown poria with tramal tissue mostly of setal hyphae and with small allantoid spores. It has long been accepted as a valid species (**1b**, p. 428; **1c**, p. 322; **2**, p. 412; **11**, p. 162; **24**, p. 19; **26**, p. 84; **27**, Nos. *148, 2105*; **29**, p. 56; **31**, p. 548).

PORIA LABYRINTHICA, Hedwigia **30**: 298. 1891

The 4 collections determined by Karsten are all alike; none, however, are dated Oct. 1891 as stated in the original description. One annotated, "Mustiala, in ligno pini 27 Sept. 1891" shows all the characters of the original description and is selected as LECTOTYPE. Egeland (**11**, p. 164) recognized it as valid but later it was considered a synonym of *P. ferrugineo-fusca* (**2**, p. 412; **8**, p. 69; **31**, p. 548). All of Karsten's specimens are *P. ferrugineo-fusca*.

PORIA LOENNBOHMII, Finska Vet.-Soc. Öfv. Förh. **46**(11): 8. 1904

The type specimen is sterile, and appears to be a resupinate condition of *Daedalea confragosa* Bolt. ex Fries which has revived several times. It differs from typical material only in that the tubes blacken in KOH.

PORIA SEPARABILIS, Soc. F. F. Fenn. Medd. **16**: 21. 1888

The type specimen is sterile. The external appearance is exactly like that of *Poria sanguinolenta* (Alb. & Schw. ex Fries) Sacc., but the hyphae are clamped. It is probably a resupinate condition of *Polyporus adustus* Willd. ex Fries.

PORIA STARBAECKII "Karst."

This appears to be an unpublished species, first mentioned by Egeland (**10a**, pp. 369–370), who quotes Bresadola's disposition of it as identical with *Polyporus rhodellus* Fries. Bourdot and Galzin (**3**, p. 669) consider it a young stage of *Poria resinascens* (Romell) Lund. & Nannf., an opinion considered doubtful by Pilát (**31**, p. 424).

The specimen in the Riksmuseet, only one known, appears to be *Poria resinascens*.

PYCNOPORUS

PYCNOPORUS MIMICUS, Trav. Sous-Sect. Troïtzk.-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geog. **8**(1): 62. 1906

The type specimen is in excellent condition, fertile, with hyaline allantoid spores 4.5–5 × 1.5 μ, and is the same as *Trametes subrosea* Weir, an opinion held by Bondarzew (**2**, p. 293), and by Dr. Ruth Macrae (letter). A later collection is the same.

Fomes cajanderi Karst. is the valid name for this plant.

PYCNOPORUS PALIBINI, Trav. Sous-Sect. Troïtzk-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geog. **12**(1 & 2): 110. 1911

The type material is a single specimen in each of two packets with the same data, dated September instead of October, as in the original description. The two pieces are alike. The plant is very poorly developed, mere knobs of fungous growth. The upper surface is somewhat brownish and appressed-woolly; pore surface dark, the pores about 3 per mm; context cream, faintly zonate, corky, the hyphae thin-walled, nonseptate, 3–5 μ in diameter; cystidia none; spores cylindrical, apiculate, 5.5–8 × 2–2.5 μ. The plant appears to be a species of *Trametes*, allied to *T. hispida* Bagl., and is not otherwise known to me.

SARCOPORIA

SARCOPORIA POLYSPORA, Hedwigia **33**: 15. 1894

The single specimen in the Karsten material is a small, much misshapen sporophore. It is abundantly fertile and appears to be the same as *Physisporus aurantiacus* var. *saloisensis* Karst.

SPONGIPELLIS

SPONGIPEL[L]IS AMBIENS, Trav. Sous-Sect. Troïtzk.-Kiakhta, Sect. pays d'Amour Soc. Imp. Russe Geog. **8**(1): 61. 1906

The type was not found. The species was placed in synonymy with *Polyporus borealis* var. *spathulata* Fries by Karsten (**23**, p. 110).

TRAMETES

TRAMETES EPILOBII, Soc. F. F. Fenn. Not. Sällsk. Förh. **9**: 361. 1868

The type is tiny, resupinate, and fertile. Additional specimens determined by Karsten are pileate and the same plant. The species is a synonym of *Trametes stereoides* Fries, as others have stated (**2**, p. 527; **3**, p. 596; **31**, p. 299; **34**, p. 24).

TRAMETES INAEQUALIS, Hedwigia **29**: 177. 1890

The two packets in the Karsten material are parts of the same collection, and the plant is a young setulose example of *Fomes robustus* Karst. Pilát (**31**, p. 508) doubtfully puts it in synonymy with *Fomes igniarius* (L. ex Fries) Kickx., and Lloyd (**25b**, p. 281) states that a collection at Upsala appears to be young nodules of *Fomes igniarius* (L. ex Fries) Kickx.

TRAMETES RIBICOLA, Soc. F. F. Fenn. Acta **11**(1): 30. 1881 (*nomen nudem*); Hedwigia **20**: 178. 1881

The type packet contains small specimens of *Polyporus rutilans* (Pers.) Fries. A later collection is the same.

Karsten early (**17**, p. 71) recognized the interrelations of this species by giving it varietal rank under *Polyporus rutilans*. Lloyd's opinion (**25b**, p. 385) that it was not a form of *P. rutilans* was based on poor specimens at Berlin.

TRAMETES SQUALENS, Rabenh.-Winter, Fungi Europ. Extraeu. Exs., Cent. 16 or 36, No. *3528*. 1886

The "types" of this species are the specimens distributed in the exsiccati. These are unfortunately not all the same species. Those at

Helsinki, Paris, Kew, and the New York Botanical Garden (2 packets) are the plant well known as *Poria albobrunnea* (Romell) Baxter, as are 8 packets so labelled by Karsten, and parts of 2 of the 3 additional packets in his herbarium. Karsten's illustration (**18c**, No. *65*) applies to the *P. albobrunnea* type of material. His comment (**20b**, p. 247) comparing *Bjerkandera roscomaculata* with it and with *Trametes serialis* Fries, indicates that he did not have a clear concept of the species. The original description is scarcely decisive, but certainly included the *P. albobrunnea* type of plant.

Another concept of the species is based on a white, narrowly reflexed species of *Polyporus* found in an unbound exsiccati packet at Helsinki, as part of 3 additional packets, and in the exsiccati packets at Upsala, Farlow Herbarium, and the Riksmuseet. The last led Romell (**36**, p. 7) to consider the species the same as *Polyporus anceps* Peck, an opinion widely accepted (**2**, p. 507; **3**, p. 592; **27**, No. *2102*; **31**, p. 312).

This reflexed white plant is unfortunately uniformly sterile. A satisfactory determination can scarcely be made, but it seems quite certain that this is not the same as *P. anceps*. The context is much too soft and fibrous for that species, and the clamped hyphae would appear to be a decisive difference. It is unknown to me.

There should be little question of Karsten's original concept of this species. Five of his exsiccati packets, his illustration, and 10 of the 11 packets in his herbarium which were collected before the publication date of his species contain the plant previously known as *Poria albobrunnea*. **Poria squalens** (Karst.) Lowe, comb. nov., is therefore considered the name prior to *P. albobrunnea*, and the bound exsiccati specimen at Helsinki is selected as the LECTOTYPE.

TRECHISPORA

TRECHISPORA ONUSTA, Hedwigia **29**: 147. 1890

This species is a synonym of *Poria candidissima* (Schw.) Cooke, according to the LECTOTYPE selected from a Karsten collection at the New York Botanical Garden by D. P. Rogers, a portion of which is now at Helsinki. This collection is the only one known which agrees with the original description, which stated that the spores were echinulate.

The specific name has long been applied to a yellow *Poria* with smooth spores. Romell (**34**, p. 11), in error, as the two plants are quite different, renamed the yellow plant *Polyporus albolutescens*. The error was subsequently recognized, for the two are uniformly separated in his herbarium at the Riksmuseet. The yellow *Poria* has long been

accepted under Karsten's name (**1a**, p. 222; **1i**, p. 228; **2**, p. 585; **3**, p. 658; **6**, p. 41 [except for spores]; **31**, p. 393; **33**, p. 80).

STATE UNIVERSITY OF NEW YORK,
COLLEGE OF FORESTRY,
SYRACUSE, NEW YORK

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UREDINALES PARASITIZING GRASSES OF THE TRIBE CHLORIDEAE [1]

JOE F. HENNEN [2] AND GEORGE B. CUMMINS

(WITH 27 FIGURES)

A taxonomic account of the 27 species of rusts (*Uredinales*) parasitizing grasses of the tribe Chlorideae throughout the world is presented in this paper. Only *Puccinia* and *Uromyces* of the six genera of rusts known to occur on grasses parasitize species of the Chlorideae. The Chlorideae and their rusts are generally tropical or subtropical in distribution. However, the distribution of a particular rust usually is not as great as that of its host or hosts. Some rust species are endemic to small areas although their hosts grow over wide areas, *i.e. Puccinia kansensis,* while other species are cosmopolitan with their hosts, *i.e. Puccinia cynodontis.* Out of a total of 27 species, 17 occur in North America, 14 in South America, and 9 in Africa. Two species are known in Europe, three in Asia, and two in Australia.

The Sydow's monograph (**20, 22**) is the only previous treatment of this group on a world-wide basis. This work included 14 of the present 27 species. Publications limited geographically have included several of the presently recognized species. Arthur (**3**) included 13 of the species of *Puccinia* in the genus *Dicaeoma* and four of the species of

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[2] Present address: Department of Plant Pathology, South Dakota State College, Brookings, South Dakota.

Uromyces in *Nigredo*. Arthur's Manual (**5**) contains descriptions of 13 of the species.

No recent taxonomic treatment of the Chlorideae is available. Therefore, Hackle's account in Engler and Prantl (**14**) and Swallen's (**20**) monograph of the North American species have been used. The tribe Chlorideae is placed by Hitchcock (**15**) in the sub-family Festucoideae. Others (**16, 19**), utilizing cytological and embryological data, place it in the sub-family Panicoideae.

This investigation substantiates the view that the genus *Beckmannia* does not belong with the Chlorideae. The following points support this view. *Puccinia graminis* and *Puccinia coronata* (*P. beckmanniae* McAlp.), according to records in the Arthur Herbarium, are restricted to the sub-family Festucoideae, with the single exception of one questionable collection of *P. graminis* on *Echinochloa crus-galli*. Collections of both of these fungi are known on *Beckmannia syzigachne*. *Uromyces beckmanniae* has long-covered, paraphysate telia, unlike other rusts on Chlorideae, but is similar to rusts on the Festuceae and Hordeae. Cytological and embryological work (**16, 19**) show that *Beckmannia* is more properly placed in the Agrostideae.

TAXONOMY

This treatment is based upon a morphological definition of species. Spermagonial and aecial stages are not described herein and are unknown for most of the species. Thus, descriptions are based only on uredia and telia. When no clear differences could be found to separate proposed taxa based on uredia and telia they were combined. When aecial stages are found they may conceivably differ enough to justify further segregation. However, aecia of heteroecious grass rusts are usually less variable than the uredial and telial stages.

Useful teliospore characters are their length and width, amount of apical wall thickening, surface markings, the length of their pedicels, and the thickness of the pedicel wall. Urediospore characters are their length and width, surface markings, number and arrangement of germ pores (Arthur (**4**) and Cummins (**9**) have pointed out the significance of this character), and amount and placement of wall thickening. The presence or absence of amphispores and paraphyses provide other characters.

In this paper epiphyllous means the adaxial leaf surface, and hypophyllous the abaxial surface.

The specimens cited are deposited in the Arthur Herbarium, Purdue University, unless otherwise noted as follows: (FH) Farlow Herbarium,

Harvard University; (UGAN) Herbarium of the Department of Agriculture of Uganda; (STOCK) Naturhistoriska Riksmuseet Botaniska Avdelningen, Stockholm; (MICH) University of Michigan Herbarium.

KEY TO GENERA

1. Teliospores usually two-celled.....1. *Puccinia*
1. Teliospores one-celled only.....2. *Uromyces*

KEY TO SPECIES OF PUCCINIA

1. Uredia with incurved marginal paraphyses (*Enteropogon, Chloris*).....1. *P. enteropogonis.*
1. Uredia without paraphyses.
   2. Urediospores echinulate.
      3. Urediospores with 3–4 equatorial germ pores.
         4. Urediospore wall thickened apically 8–10 μ, teliospores 21–27 × 37–64 μ (*Spartina*).....2. *P. sparganioides.*
         4. Urediospore wall evenly thickened, teliospores 19–25 × 26–39 μ.
            5. Teliospore pedicels thick-walled, not collapsing, urediospores with only equatorial pores (*Bouteloua, Chloris*).....3. *P. cacabata.*
            5. Teliospore pedicels thin-walled, collapsing laterally, urediospores sometimes with both equatorial and scattered germ pores (*Gouinia*).....4. *P. guaranitica.*
      3. Urediospores with 4–10 scattered germ pores, or pores indistinct.
         6. Teliospores visibly bilamellate throughout, side wall 3–5 μ thick, golden to clear chestnut-brown (*Gymnopogon*).....5. *P. gymnopogonicola.*
         6. Teliospores visibly bilamellate only in apical portions, side wall usually not over 3 or 4 μ thick, chestnut-brown.
            7. Urediospores with apical wall thickened 3–15 μ.
               8. Teliospores 15–26 × 35–55 μ, urediospore wall thickened irregularly (*Spartina*).....6. *P. seymouriana.*
               8. Teliospores 16–24 × 24–35 μ, urediospore wall thickened only apically (*Chloris, Dactyloctenium*).....7. *P. dietelii.*
            7. Urediospores with evenly thickened walls.
               9. Teliospore pedicels thick-walled, not collapsing laterally.
                  10. Tuberculate amphispores always produced (*Bouteloua*).....8. *P. vexans.*
                  10. Amphispores not produced.
                     11. Teliospores 17–26 × 24–31 μ (*Bouteloua*)...9. *P. exasperans.*
                     11. Teliospores 20–27 × 30–39 μ (*Schedonnardus*).....10. *P. schedonnardi.*
               9. Teliospore pedicels thin-walled, usually collapsing laterally.
                  12. Teliospore pedicels less than 70 μ long.
                     13. Urediospores 23–28 × 26–33 μ, teliospores 21–27 × 42–64 μ, wall thick apically (*Spartina*).....11. *P. distichlidis.*
                     13. Urediospores 12–18 × 13–22 μ, teliospores 16–22 × 24–32 μ, wall uniform (*Buchloë*).....12. *P. kansensis.*

12. Teliospore pedicels up to 90, 100, or 120 μ long.
14. Teliospores with septum mainly vertical, *i.e.* diorchidioid (*Bouteloua*) ..... 13. *P. bouteluae.*
14. Teliospores with septum horizontal.
15. Urediospores 16–23 × 18–28 μ.
16. Apical wall of teliospore 4–9 μ thick (*Chloris, Bouteloua, Leptochloa, Trichloris*) ... 14. *P. chloridis.*
16. Apical wall of teliospore 2–4(–7) μ thick (*Gouinia*) ..... 4. *P. guaranitica.*
15. Urediospores 13–15 × 16–18 μ (*Leptochloa*) ..... 15. *P. subtilipes.*

2. Urediospores verrucose.
17. Urediospore germ pores 3, equatorial (*Cynodon*) ..... 16. *P. cynodontis.*
17. Urediospore germ pores 6–8, scattered or bizonate.
18. Telia mostly hypophyllous, urediospore walls 1.5–2.5 μ, light golden to light cinnamon-brown (*Leptochloa*) ..... 17. *P. leptochloae.*
18. Telia mostly epiphyllous, urediospore walls 2–3 μ thick, dark cinnamon-brown (*Bouteloua*) ..... 18. *P. opuntiae.*

DESCRIPTIONS OF SPECIES

1. PUCCINIA ENTEROPOGONIS Syd., Monog. Ured. **1**: 751. 1904 (FIG. 1).

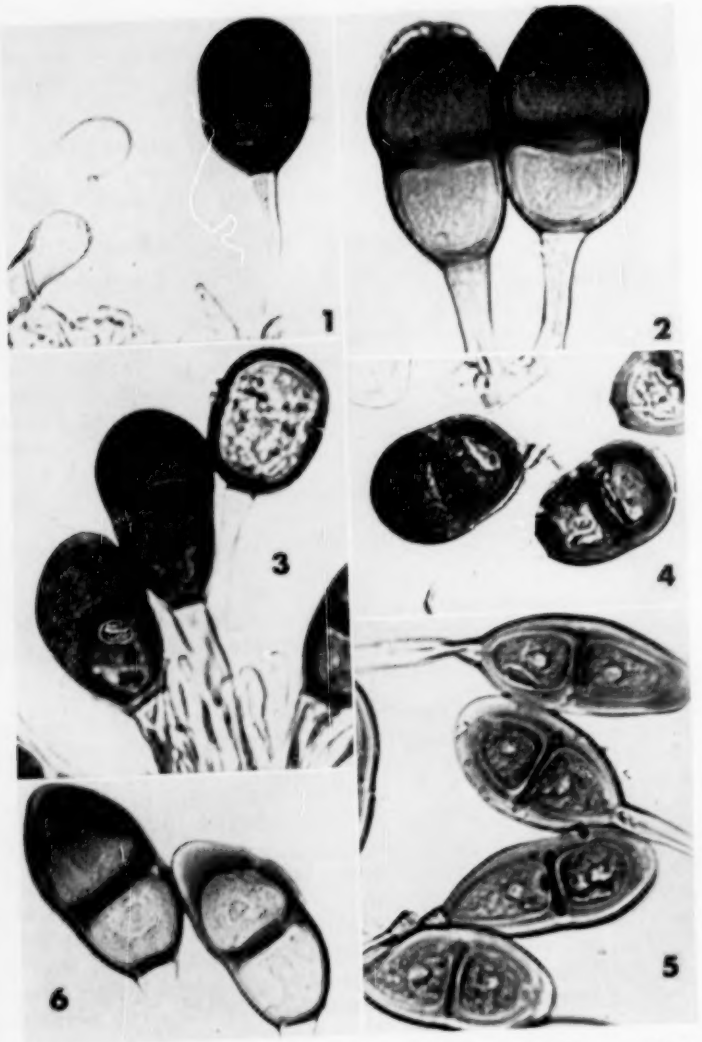
Dicaeoma enteropogonis (Syd.) Syd., Ann. Myc. **20**: 117. 1922.
Puccinia chloridis-incompletae Ramakrishnan, Srinivasan, & Sundaram in Proc. Indian Acad. Sci. B. **36**: 91. 1952.

Spermogonia and aecia unknown. Uredia chiefly hypophyllous, scattered, cinnamon-brown, broadly elliptical, 0.1–0.3 mm long; paraphyses peripheral, variable, mostly 1-septate, cylindric to clavate, usually incurved, often geniculate, 7–9 × 24–47 μ; wall hyaline to light golden, 2 μ thick; urediospores globose to ovoid, 18–20 × 19–25(–28) μ; wall cinnamon-brown, 1.5 μ thick, echinulate, pores 6–8, scattered. Telia chiefly hypophyllous, scattered, blackish brown, circular to oblong, 0.1–1.0 mm long, pulvinate; teliospores oblong-ellipsoid, or approaching globoid, rounded above and below, not constricted at the septum, 18–24 × 24–32 μ; wall evenly dark chestnut-brown, 1.5–2.5 μ thick at sides, 2.5–4(–6) μ thick at apex, smooth; pedicel light golden to light brown, thick-walled, usually not collapsing laterally, up to 130 μ long, persistent.

HOSTS AND DISTRIBUTION: *Enteropogon monostachyus* (Vahl) K. Schum., *Chloris incompleta* Roth; India, Tanganyika, Zanzibar.

TYPE: Usambara, Tanganyika, 1893, *C. Holst*, on *Enteropogon monostachyus* (Vahl) K. Schum.

ILLUSTRATIONS: Sydow, Ann. Myc. **20**: pl. 40, f. 528.



FIGS. 1–6. 1. Paraphyses and teliospore of *Puccinia enteropogonis* (from type). 2. Teliospores of *Puccinia sparganioides* (from type). 3. Teliospores and urediospore of *Puccinia cacabata* (from type). 4. Teliospores of *Puccinia guaranitica* (from type). 5. Teliospores of *Puccinia gymnopogonicola* (from type). 6. Teliospores of *Puccinia seymouriana* (from type). All × ca. 800.

SPECIMENS EXAMINED: On *Enteropogon monostachyus*, TANGANYIKA: Usambara, 1893, *C. Holst;* ZANZIBAR: Aug. 27, 1929, *A. S. Hitchcock 24438;* on *Chloris incompleta*, INDIA: Kalar (Coimbatore), Jan. 26, 1952, *N. U. Sundaram* (TYPE of *P. chloridis-incompletae*).

This species is particularly distinctive because of the septate paraphyses. No other species on the Chlorideae has uredia with paraphyses, and septate paraphyses are rare in *Puccinia*.

2. PUCCINIA SPARGANIOIDES Ell. & Barth., Erythea **4**: 2. 1896 (FIG. 2).

Aecidium fraxini Schw., Schr. Nat. Ges. Leipzig **1**: 66. 1822.
Caeoma fraxinatum Link in Wild. Sp. Pl. **6**(2): 62. 1825.
Caeoma (Roestelia) fraxinites Schw., Trans. Am. Phil. Soc. II. **4**: 294. 1832.
Uredo peridermiospora Ell. & Tracy, Jour. Myc. **6**: 77. 1890.
Puccinia peridermiospora Arth., Science II. **10**: 565. 1899.
Puccinia fraxini Arth., Bot. Gaz. **34**: 6. 1902. Not *P. fraxini* Koern. 1898.

The aecial stage, which occurs on species of *Forestiera* and *Fraxinus*, is described by Arthur (**3**, **5**) and a record of cultures is summarized by Arthur (**5**).

Uredia chiefly hypophyllous (epiphyllous on *Spartina alterniflora* var. *glabra*), crowded and often confluent, ellipsoid or oblong-linear, 0.5–5 mm long, pulverulent, pale yellow, surrounded by the ruptured epidermis; urediospores obovoid, ellipsoid or oblong, 18–30 × 29–47 μ; wall colorless, 1.5–3 μ thick at sides, 8–10 μ thick at apex, echinulate, pores 4, equatorial, sometimes covered with noticeable umbos. Telia usually hypophyllous, also on inflorescences, oblong-linear, 1–5 mm long (up to 3.5 cm on sheaths and culms), surrounded by ruptured epidermis, compact, pulvinate, blackish brown, teliospores oblong or ellipsoid, rounded at both ends or obtuse above, narrowed below, slightly constricted at the septum, 14–24 × 37–64 μ; wall chestnut-brown, lighter in lower cell, 1.5 μ thick at sides, 5–7 μ thick at apex, smooth; pedicel pale yellow or hyaline, wall sometimes thickened, usually collapsing laterally, up to 100 μ long, persistent.

HOSTS AND DISTRIBUTION: *Spartina alterniflora* Loisel, *S. bakeri* Merr., *S. brasiliensis* Raddi, *S. cynosuroides* (L.) Roth, *S. gracilis* Trin., *S. patens* (Ait.) Muhl., *S. pectinata* Link; Brazil, Canada, U. S. A.

TYPE: Rooks Co., Kans., Aug. 24, 1895, *E. Bartholomew*, on *Spartina pectinata*, mistaken for *Carex sparganioides*.

ILLUSTRATIONS: Arth., Bot. Gaz. **34**: 9, f. 2f, g, h; Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 5, 6, f. 54a–k; Arth., Man. Rusts, f. 178.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

The name *Puccinia sparganioides* is reinstated for this species in order to conform with the code of nomenclature (**17**).

The aecial stage causes distortions on young leaves. Some cases of complete defoliation of ash have been reported in South Dakota (**7**).

Characteristic features of this species are the apically thickened urediospores, which closely resemble the aeciospores, and the hypophyllous uredia and telia. The uredia differ in the latter respect when they occur on *S. alterniflora* var. *glabra* by being epiphyllous.

This species is similar to *P. seymouriana*. Even though the teliospores are indistinguishable morphologically, the telia of *P. seymouriana* are consistently epiphyllous while those of *P. sparganioides* are either hypophyllous or on the sheaths, culms, or inflorescences. The urediospores of the two species are similar, both having apical thickenings and sparsely placed echinulations, but the aeciospores differ.

The aecia occurring upon *Forestiera* in Florida and Mexico are typical; however, the aeciospores from *Forestiera angustifolia* from Texas lack the usual apical thickenings.

3. PUCCINIA CACABATA Arth. & Holw. in Arth., Proc. Am. Phil. Soc. **64**: 179. 1925 (FIG. 3).

Aecidium gossypii Ell. & Ev., Erythea **4**: 3. 1896.
Uredo chloridis-berroi Speg., Rev. Argent. Bot. **1**: 135. 1925.
Puccinia stakmanii Presley in Presley & King, Phytopathology **33**: 385. 1943.
Uredo chloridis-polydactylidis Viegas, Bragantia **5**: 82. 1945.

The aecial stage occurs on species of *Gossypium* in northern Mexico and the Southwestern United States. For a description see Ellis & Everhardt and Presley & King.

Uredia mostly epiphyllous on *Bouteloua* sp., amphigenous on *Chloris* sp., oblong or linear, becoming confluent, 0.3 to several mm long, cinnamon-brown, early naked, pulverulent, the ruptured epidermis apparent; urediospores globoid, broadly ellipsoid or obovoid, 19–24 × 23–31 μ; the wall pale to dark cinnamon-brown, 1.5–2.5 μ thick, moderately echinulate, the pores 3 or rarely 4, equatorial, distinct. Telia

amphigenous and caulicolous, often crowded, rounded, elliptic, or linear, becoming confluent, pulvinate, 0.3 to several mm long, early naked, chocolate-brown, ruptured epidermis at first conspicuous, teliospores variable in form, size, and color, ellipsoid, oblong, or broadly ellipsoid, 19–26 × 27–39 μ, rounded at both ends or somewhat obtuse above, not or slightly constricted at the septum, the septum sometimes oblique or vertical; wall dark chestnut-brown, the oblong and thinner-walled spores usually paler, 2–4 μ at sides, 4–9 μ thick at the apex, smooth; pedicel up to 90 μ long, hyaline or light golden throughout, thick-walled, not collapsing, persistent.

HOSTS AND DISTRIBUTION: *Bouteloua aristidoides* (H.B.K.) Griseb., *B. barbata* Lag., *B. parryi* (Fourn.) Griff., *B. rothrockii* Vasey, *Chloris berroi* Arech., *C. ciliata* Sw., *C. polydactyla* (L.) Sw., *Chloris* sp.; Argentina, Bahama Islands, Bolivia, Brazil, Dominican Republic, Mexico, Santo Domingo, U. S. A.

TYPE: Sur Yungas, Bolivia, hacienda Anacuri, June 5, 1920, *E. W. D.* and *Mary M. Holway* (Reliquiae Holwayanae No. 88), on *Chloris ciliata*.

ILLUSTRATIONS: Presley & King, Phytopath. **33**: 385, f. 2.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

Presley and King demonstrated that cultivated cotton and ten wild species of *Gossypium* were susceptible to infection by *P. stakmanii* (= *cacabata*) by artificial inoculation. Most of these wild species of *Gossypium* occur as endemics in the regions where the rust has been found on *Bouteloua*.

P. cacabata is recognized by its echinulate, cinnamon-brown urediospores with equatorial pores. The teliospores resemble those of *P. exasperans*, *P. vexans*, and *P. opuntiae* in having thick-walled noncollapsing pedicels.

4. PUCCINIA GUARANITICA Speg., An. Soc. Ci. Argent. **26**: 12. 1888 (FIG. 4).

Dicaeoma guaraniticum (Speg.) Kuntze, Rev. Gen. **3**(3): 471. 1898.
Puccinia chichenensis Mains, Carnegie Inst. of Washington Publ. 461: 100. 1935.

Spermogonia and aecia unknown. Uredia amphigenous, scattered, linear, 0.5–1 mm long, pulverulent, cinnamon-brown, surrounded by ruptured epidermis; urediospores broadly ellipsoid to obovoid, 17–23 × 20–

28 μ; wall golden to cinnamon-brown, 2–4 μ thick, echinulate, pores 4–6, scattered, or 4 or 5 equatorial. Telia amphigenous, scattered, 0.3–0.5 mm long, blackish brown, pulvinate, surrounded by ruptured epidermis; teliospores broadly ellipsoid, rounded at both ends, not constricted at the septum, 20–25 × 28–32 μ; wall chestnut-brown, 2–4 μ thick at sides, 4–7 μ thick at apex, smooth; pedicel uniformly golden, thin-walled and collapsing laterally, up to 100 μ long, persistent.

HOSTS AND DISTRIBUTION: *Gouinia guatamalensis* (Hack.) Swallen, *G. latifolia* (Griseb.) Vasey, *G. ramosa* Swallen; Bolivia, Honduras, Mexico, Paraguay.

TYPE: Guarapi, Paraguay, Oct., 1888, *B. Balansa*, on *Tricuspis latifolia* Griseb. (*Gouinia latifolia*).

ILLUSTRATIONS: Syd. Monog. Ured. **1**: pl. 43, f. 554.

SPECIMENS EXAMINED: On *Gouinia guatamalensis*, HONDURAS: road between El Jicarito and El Pedregal, Dept. of Morazan, Nov. 13, 1948, *Standley;* on *G. latifolia*, BOLIVIA: Chulumani, Sur Yungas, Dec. 22, 1923, *Hitchcock 22650½* (MICH); PARAGUAY: Guarapi, Oct., 1888, *Balansa* (TYPE); on *G. ramosa*, MEXICO: Chichen Itza, Yucatan, July 7–13, 1932, *Swallen 2484* (TYPE of *Puccinia chichenensis*).

No significant differences exist between *Puccinia guaranitica* and *P. chichenensis*.

This species is particularly interesting because urediospores with both equatorial and scattered germ pores occur.

5. **Puccinia gymnopogonicola** Hennen, sp. nov. (FIG. 5).

Spermogoniis et aecidiis ignotis. Urediis epiphyllis, sparsis, 0.5–3 mm longis, flavidis; urediosporis globoideis vel late ovatis, 13–16 × 16–22 μ; membrana hyalina vel flavida, echinulata, 1.5–2 μ crassa, poris germ. indistinctis. Teliis amphigenis, plerumque hypophyllis, atro-brunneis, 0.5–2 mm longis; teliosporis late ellipsoideis vel oblongis, medio non vel vix constrictis, (16–)18–22 × (26–)28–41 μ; membrana atro-aurea vel liquidius castanea, conspicue bilamellata, 3–5 μ crassa, ad apicem 4–6 μ crassa, levi; pedicello hyalino vel fulvo, usque 80 μ longo, persistenti.

Spermogonia and aecia unknown. Uredia epiphyllous, scattered, 0.5–3 mm long, yellow, ruptured epidermis not apparent; urediospores small, ovoid to nearly globoid, 13–16 × 16–22 μ; wall hyaline or yellowish, finely echinulate, 1.5–2 μ thick, pores obscure. Telia amphigenous, chiefly hypophyllous, blackish brown, broadly elliptic, pulvinate, 0.5–2 mm long, sometimes confluent; teliospores broadly ellipsoid to oblong, slightly or not constricted at the septum, rounded at both ends or tapering toward apex and base, (16–)18–22 × (26–)28–41 μ; wall deep golden to clear chestnut, visibly bilamellate throughout, 3–5 μ thick at

sides, 4–6 μ thick at apex, smooth; pedicels hyaline to brownish, narrow, thin-walled, usually collapsing laterally, up to 80 μ long, persistent.

HOSTS AND DISTRIBUTION: *Gymnopogon burchellii* (Munro) Ekman, *G. spicatus* (Spr.) Ktze.; Brazil.

TYPE: Mandaqui, Sao Paulo, Brazil, May 25, 1922, *E. W. D.* and *Mary M. Holway 1888* (Reliquiae Holwayanae No. *146*, as *Puccinia gymnopogonis*), on *Gymnopogon burchellii*.

SPECIMENS EXAMINED: On *Gymnopogon burchellii*, BRAZIL: Campos do Jordao, Sao Paulo, Apr. 27, 1922, *Holway 1779* (Reliq. Holw. No. *142*, as *Puccinia gymnopogonis*); Mandaqui, Sao Paulo, May 25, 1922, *Holway 1888* (Reliq. Holw. No. *146*, as *Puccinia gymnopogonis*, TYPE); on *G. spicatus*, Campo Grande, Matto Grosso, Feb. 7–11, 1930, *Chase 10827*; Lagoa Santa, Bello Horizonte, Minas Geraes, Mar. 23–24, 1925, *Chase 9051*.

Previously these specimens had been identified as *P. gymnopogonis*, whose type, along with several other specimens, belongs with *P. boutelouae* because of the diorchidioid teliospores. *P. gymnopogonicola* is wholly unlike *P. boutelouae*. The distinguishing character is the thick, golden to clear chestnut-brown teliospore wall, which shows a distinct lamellation throughout.

6. PUCCINIA SEYMOURIANA Arth., Bot. Gaz. **34**: 11. 1902 (FIG. 6).

Aecidium cephalanthi Seym. in Burrill, Bot. Gaz. **9**: 191. 1884.
Aecidium obesium Arth., Bull. Torrey Bot. Club **37**: 579. 1910.
Puccinia cephalanthi Barth., N. Am. Ured. No. *261* emend. 1922.

The aecial stage, which occurs on species of *Cephalanthus*, *Asclepias*, *Apocynum*, and *Amsonia*, has been described by Arthur (**3**, **5**) and a summary of cultures is given by Arthur (**5**).

Uredia epiphyllous, narrowly oblong, or oblong-linear, 0.5–1 mm long, early naked, ruptured epidermis inconspicuous, pulverulent, orange-yellow when fresh; urediospores obovoid or broadly ellipsoid, 19–27 × 29–41 μ; wall hyaline to yellowish, 2–3 μ thick at sides, 9–15 μ thick at apex, echinulate, pores obscure. Telia epiphyllous, usually numerous, crowded, sometimes confluent, oblong, pulvinate, compact, 0.5 mm broad by 1–5 mm long, early naked, ruptured epidermis not apparent, chocolate-brown; teliospores oblong, cylindric, or broadly ellipsoid, 15–26 × 35–55 μ, rounded, obtuse, or acute above, sometimes narrowed at the base, very slightly constricted at the septum; wall chestnut or dark chestnut-brown, usually lighter below, about 1.5 μ thick laterally, 5–9 μ apically, smooth; pedicel colorless, up to 100 μ long, sometimes thick-walled and non-collapsing, or collapsing basally, persistent.

HOSTS AND DISTRIBUTION: *Spartina bakeri* Merr., *S. cynosuroides* (L.) Roth, *S. pectinata* Link, *S. spartinae* (Trin.) Merr.; U. S. A.

TYPE: Racine, Wis., Sept. 10, 1899, *J. J. Davis*, on *Spartina pectinata* (Arth. & Holw., Ured. Exsic. et Ic., No. *53a*).

ILLUSTRATIONS: Arth., Bot. Gaz. **34**: 13, f. 3k, l; Arth. & Holw., Bull. Lab. Nat. Hist. Iowa **5**: pl. 4, f. 53a, b; Arth., Man. Rusts, f. 179.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive but are listed in the original thesis filed in the Purdue University Library.

The resemblance between the aeciospores and urediospores is even more striking than in *P. sparganioides*. This resemblance gave Arthur the idea that the *Aecidium* on *Cephalanthus* was the aecial stage of the rust, which he proved by cultures. According to Arthur (**5**), three races or varieties were shown by cultures, corresponding to the three families of aecial hosts; however, no morphological differences have been detected.

The relationship between this species and *P. sparganioides* is discussed under that species.

One specimen in the Arthur Herbarium collected on *Spartina spartinae* from between Houston and Galveston, Texas, differs from collections on *S. pectinata*. The uredia and telia occur deeper within the intercostal grooves, the urediospores have irregularly thickened walls, sometimes forming a stellate cavity, and do not have the typical apical thickenings.

7. PUCCINIA DIETELII Sacc. & Syd. in Sacc., Syll. Fung. **14**: 358. 1899 (FIG. 7).

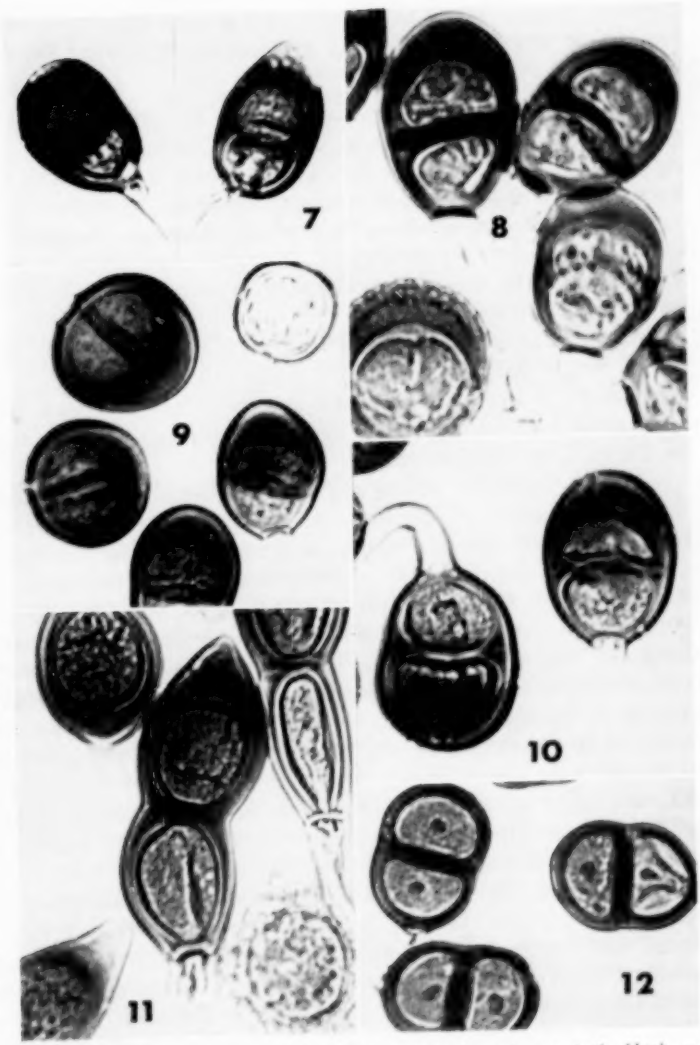
Puccinia chloridis Diet., Hedwigia **31**: 290. 1892. Not *P. chloridis* Speg. 1891.

Puccinia chloridina Bacc., Ann. Bot. **4**: 269. 1906.

Puccinia chloridicola P. Henn. in Fl. Bas-et Moy.-Congo, Ann. Mus. Congo **2**(2): 90. 1907.

Puccinia dactyloctenii Pat. & Har., Bull. Soc. Myc. Fr. **24**: 13. 1908.

Spermogonia and aecia unknown. Uredia amphigenous, scattered, narrowly oblong, 0.3–1.0 mm long, yellowish to brownish; urediospores ellipsoid or obovoid, 15–21 × 17–26 μ; wall golden to hyaline, echinulate, 1.5–2 μ thick at sides, 3–10 μ at apex, the pores indistinct. Telia amphigenous, scattered, round or elliptic, pulvinate, 0.3–0.5 mm long, ruptured epidermis not apparent, pulverulent, dark chocolate-brown; teliospores short oblong to broadly ellipsoid, 17–24 × 24–35 μ, usually rounded at both ends, not or slightly constricted at the septum; wall



FIGS. 7–12. 7. Teliospores of *Puccinia dictelii* (from Long, Arth. Herb. acces. no. *22754*). 8. Teliospores and amphispore of *Puccinia vexans* (from type). 9. Teliospores and urediospore of *Puccinia exasperans* (from type). 10. Teliospores of *Puccinia schedonnardi* (from type). 11. Teliospores and urediospore of *Puccinia distichlidis* (from type). 12. Teliospores of *Puccinia kansensis* (from type). All × ca. 800.

dark chestnut-brown, 2–3 μ thick at sides, 5–7 μ thick at apex, smooth; pedicel golden, more deeply colored next to spore, thin-walled and collapsing laterally, up to 75 μ long, persistent.

HOSTS AND DISTRIBUTION: *Chloris abyssinica* Hochst., *C. chlorideae* (Presl.) Hitchc., *C. cuculata* Bisch., *C. gayana* Kunth, *C. latisquamea* Nash, *C. radiata* Sw., *C. virgata* Sw., *C. verticilliata* Nutt., *Chloris* sp., *Dactyloctenium aegypticum* (L.) Beauv.; Abyssinia, Ambatocam, Argentina, French Congo, Mexico, Nyassaland, Rhodesia, Tanganyika, Uganda, U. S. A.

TYPE: Rooks Co., Kans., Mar. 23, 1892, *E. Bartholomew* No. *526*, as *Puccinia chloridis* Diet.

ILLUSTRATIONS: Arth. & Holway, Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 7, f. 56a, b; Arth., Man. Rusts, f. 183.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

The species, previously known as *Puccinia chloridis* Speg., has been divided into two parts. Collections with no apically thickened urediospores, including the type and all but one other South American collection, have been united with what has been known as *Puccinia bartholomaei* Diet. in North America. This group retains the name, *P. chloridis* Speg. The remaining collections of the old *P. chloridis* are from North America and Africa and have apically thickened urediospores. Dietel first described this taxon as *P. chloridis* but the epithet had previously been used by Spegazzini. Saccardo and Sydow recognized that the rusts named by Spegazzini and by Dietel were different and gave the name *dietelii* to the latter.

Examination of the types of *P. chloridina* and *P. dactyloctenii* showed they also belonged here. Material of *P. chloridicola* has not been seen but the rust is probably the same.

The outstanding feature of this species is the apical thickening of the urediospores. This character separates it from *P. chloridis*. Cultures and field collections of *P. bartholomaei* (= *Chloridis*) on *Asclepias* show that aeciospores of *P. chloridis* have apical thickenings similar to those of urediospores of *P. dietelii*. This may indicate that these rusts are closely related and that *Asclepias* may serve as an aecial host of *P. dietelii*.

8. PUCCINIA VEXANS Farl., Proc. Am. Acad. **18**: 82. 1833 (FIG. 8).

Uromyces brandegei Peck, Bot. Gaz. **4**: 127. 1879.

Puccinia aristidicola P. Henn., Hedw. **35**: 243. 1886.
Dicaeoma vexans (Farl.) Kuntze, Rev. Gen. **3**(3): 471. 1898.

Spermogonia and aecia unknown. Uredia amphigenous, oval or oblong, pulverulent, cinnamon-brown, ruptured epidermis inconspicuous; urediospores globoid or broadly ellipsoid, 23–29 × 26–30 μ, wall cinnamon-brown, 2–3.5 μ thick, strongly echinulate, pores 8, scattered; amphispores and teliospores usually intermixed or amphispores alone, sori amphigenous, circular or broadly ellipsoid, blackish brown, pulvinate, ruptured epidermis not apparent, amphispores obovoid, 26–35 × 34–42 μ, the wall 3–4 μ thick at sides, 7–12 μ at apex, distinctly verrucose-tuberculate, dark chestnut-brown, the pores 3–4, equatorial; the pedicel usually persistent, hyaline, thick-walled and non-collapsing; teliospores broadly ellipsoid or globoid, (19–)23–29 × 32–40 μ, rounded at both ends, usually not constricted at the septum; wall evenly chestnut-brown, 2.5–3 μ thick at sides, 6–8 μ thick at apex, smooth; pedicel hyaline, up to 95 μ long, thick-walled, usually not collapsing, persistent.

HOSTS AND DISTRIBUTION: *Bouteloua breviseta* Vasey (?), *B. curtipendula* (Michx.) Torr.; Argentina, Bolivia, Mexico, Peru, U. S. A.

TYPE: Decorah, Iowa, *E. W. D. Holway* (Ellis, N. Am. Fungi, No. *1051*), on *Bouteloua curtipendula*.

ILLUSTRATIONS: Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 8, f. 58a–e; Arth., Bull. Torrey Bot. Club **32**: 37, f. 1; Carleton, Bull. Plant Industry 63: pl. 2, f. 2–9; Freeman, Minn. Pl. Diseases 165, f. 77; Arth., Jour. Appl. Micr. **5**: 1656, f. 3; Arth., Man. Rusts, f. 185

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive but are listed in the original thesis filed in the Purdue University Library.

The type was distributed as No. *1051* of Ellis's N. Am. Fungi. The collection from the Farlow Herbarium was examined and found to have both *P. chloridis* and *P. vexans* growing on the same leaf. These two species are distinguished by the fact that amphispores are always present in telia of *P. vexans* and never in *P. chloridis*. Teliospore pedicels are thick-walled and non-collapsing in *P. vexans* but thin-walled and collapsing in *P. chloridis*. Urediospores measure 23–29 × 26–30 μ in *P. vexans*, are cinnamon-brown and have easily visible pores. Urediospores measure 16–22 × 18–23 μ in *P. chloridis*, are usually hyaline and have obscure pores.

The dark chestnut-brown, tuberculate amphispores are always produced by this species and are the most characteristic feature because no other rust of Chlorideae has such spores. Their occurrence in the rusts has been discussed by Arthur (**1**).

Amphispores of *P. vexans* have been germinated by Carleton (**8**) and Arthur (**2**). The latter used them in culture work and proved their ability to infect the telial host.

Davis (**12**) reported culturing *P. vexans* on *Asclepias tuberosa* and *A. syriaca*. However, examination of this material proved that the teliospores used were *P. chloridis* (*P. bartholomaei*).

9. PUCCINIA EXASPERANS Holw., Ann. Myc. **3**: 21. 1905 (FIG. 9).
Dicaeoma exasperans (Holw.) Arth. & Fromme, N. Am. Flora **7**: 319. 1920.

Spermogonia and aecia unknown. Uredia amphigenous, rounded or elliptical, cinnamon-brown, surrounded by ruptured epidermis, pulverulent; urediospores globose, ovoid, or broadly elliptical, 17–25 × 22–29 μ; wall cinnamon-brown, 1.5–2 μ thick, echinulate, pores 6–8, scattered or bizonate. Telia amphigenous, blackish, rounded to broadly elliptical, 0.25–0.5 mm long, ruptured epidermis not apparent; teliospores globoid to broadly ellipsoid, 17–26 × 24–31 μ, arising from chestnut-brown basal cells, often diorchidioid, rounded at both ends, not constricted at the septum; wall chestnut-brown, 1.5–3 μ thick laterally, 4–10 μ thick apically, smooth; pedicel thick-walled, not collapsing, hyaline or golden, up to 125 μ long, persistent.

HOSTS AND DISTRIBUTION: *Bouteloua disticha* (H.B.K.) Benth., *B. curtipendula* (Michx.) Torr., *B. pringlei* Scribn., *B. triathera* Benth.; Mexico, U. S. A.

TYPE: Cuernavaca, Morelos, Mexico, Oct. 30, 1903, *E. W. D. Holway* No. *5280* on *Bouteloua curtipendula*.

SPECIMENS EXAMINED: On *Bouteloua disticha*, MEXICO: Chiripitiro and Coyuca, Guerrero, Oct. 26, 1924, *Hinton 6871*; on *B. curtipendula*, MEXICO: Cuernavaca, Morelos, Oct. 30, 1903, *Holway 5280* (TYPE); U. S. A.: Lincoln, Neb., Oct. 20, 1941, *Presley*; Denton, Tex., Oct. 28, 1907, *Long* (Barth., Fungi Columb., No. *2654*, as *Puccinia boutelouae*); Denton, Tex., Sept. 16, 1951, *Hennen*; Brownwood, Tex., Sept. 28, 1950, *Cory*; College Station, Tex., Oct. 10, 1949, *Whitehead*; on *B. pringlei*, MEXICO: Iguala, Guerrero, Oct. 24, 1900, *Pringle 8374*; on *B. triathera*, MEXICO: Rio Balsas, Guerrero, Dec. 14, 1938, *Lyonnet*.

Some collections of urediospores of this species were previously thought to be amphispores of *P. boutelouae*; however, no amphispores are produced by either species. Both species have been found parasitizing the same leaf blades.

P. exasperans was included as a synonym of *P. boutelouae* by Arthur (**5**). It is distinguished from this species by its thick-walled, non-

collapsing teliospore pedicels and cinnamon-brown urediospores. It is similar to *P. vexans*, except for the lack of amphispores and the smaller urediospores and teliospores.

10. PUCCINIA SCHEDONNARDI Kell. & Swing., Jour. Mycol. **4**: 95. 1888 (FIG. 10).

Caeoma (Aecidium) hibisciatum Schw., Trans. Am. Phil. Soc. II. **4**: 293. 1832.
Aecidium (Caeoma) hibisciatum Schw., Trans. Am. Phil. Soc. II. **4**: 309. 1832.
Aecidium napaeae Arth. & Holw. in Arth., Bull. Iowa Agr. Coll. Dept. Bot. **1884**: 166. 1884.
Puccinia windsoriae Burr., Bull. Ill. Lab. Nat. Hist. **2**: 197. 1885. Not *P. windsoriae* Schw. 1832.
Aecidium calirhoes Ell. & Kell., Jour. Mycol. **2**: 4. 1886.
Aecidium malvastri Ell. & Tracy, Jour. Mycol. **7**: 43. 1881.
Aecidium sphaeralceae Ell. & Ev., Bull. Torrey Bot. Club **22**: 364. 1895.
Puccinia triodiae Ell. & Barth., Erythea **4**: 3. 1896.
Dicaeoma schedonnardi (Kell. & Swing) Kuntze, Rev. Gen. **3**(3): 470. 1898.
Dicaeoma triodiae Kuntze, Rev. Gen. **3**(3): 471. 1898.
Puccinia epicampes Arth., Bull. Torrey Bot. Club **28**: 662. 1901.
Puccinia muhlenbergiae Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: 317. 1902.
Puccinia hibisciata Kell., Jour. Myc. **9**: 110. 1903.
Aecidium malvicola Arth., Bull. Torrey Bot. Club **31**: 6. 1904.
Dicaeoma muhlenbergiae (Arth. & Holw.) Arth., Proc. Ind. Acad. Sci. **1903**: 148. 1904.
Dicaeoma hibisciatum (Schw.) Arth., Result. Sci. Congr. Bot. Vienne 344. 1906.
Puccinia subglobosa Speg., An. Mus. Nac. Buenos Aires **19**: 300. 1909. Not *P. subglobosa* Diet. & Holw., 1901.
Puccinia spegaziniella Sacc. & Trav. in Sacc., Syll. Fung. **20**: 627. 1911.

The aecial stage, which occurs on species of *Sphaeralcea*, has been described by Arthur (**3**, **5**) and a summary of cultures is given by Arthur (**5**). *Aecidium gossypii* Ell. and Ev., usually assigned here, belongs to *Puccinia cacabata*.

Uredia amphigenous, few, oblong or linear, 0.3–0.7 mm long, early naked, the ruptured epidermis not apparent, pulverulent, light cinnamon-brown; urediospores globoid to obovoid, 18–22 × 19–24 μ; wall 1.5–2.5 μ thick, echinulate, light cinnamon-brown; the pores 6–8, scattered. Telia amphigenous, numerous, often crowded, rounded, elliptic, or linear, 0.3–2 mm long, pulvinate, dark chocolate-brown, early naked, ruptured epidermis inconspicuous; teliospores broadly ellipsoid or short oblong, 20–27 × 30–39 μ, rounded at both ends, not constricted at the septum; wall dark chestnut-brown, 2–3 μ thick laterally, 4–7 μ thick apically, smooth; pedicel evenly light golden, up to 80 μ long, pedicel thick-walled, usually not collapsing laterally.

HOSTS AND DISTRIBUTION: *Schedonnardus paniculatus* (Nutt.) Trel.; U. S. A. In addition to this uredial and telial host in the Chlorideae, *Puccinia schedonnardi* has aecial stages on *Abutilon, Callirhoe, Hibiscus, Malvastrum, Napaea, Sida,* and *Sidalacea,* and uredial and telial stages on *Linnodea, Muhlenbergia* and *Sporobolus* of the Agrostideae, and *Melica, Monanthochloe,* and *Triplasis* of the Festuceae.

TYPE: Manhattan, Kans., Mar., 1888, *W. A. Kellerman* and *W. T. Swingle* No. *1268,* on *Schedonnardus texanus,* now *S. paniculatus.*

ILLUSTRATIONS: Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 2, 3, f. 50a–e; pl. 7, f. 57a, b; Arth., Man. Rusts, f. 158; Syd., Monog. Ured. **1**: pl. 42, f. 546.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

This species has the largest telial host range of any of the rusts on the Chlorideae. Most of these hosts are in the Agrostideae and Festuceae.

11. PUCCINIA DISTICHLIDIS Ell. & Ev., Proc. Acad. Phila. **1893**: 152. 1893 (FIG. 11).

Dicaeoma distichlidis (Ell. & Ev.) Kuntze, Rev. Gen. **3**(3): 468. 1898.

Puccinia kelseyi Syd., Monog. Ured. **1**: 806. 1904.

The aecial stage, which occurs on species of *Lysimachia* and *Glaux,* has been described by Arthur (**3, 5**) and who has also (**5**) summarized the cultures.

Uredia epiphyllous, intercostal, oval, numerous, yellowish, ruptured epidermis conspicuous; urediospores globoid or broadly ellipsoid, 23–28 × 26–33(–35) μ; wall pale yellow, evenly 3–4 μ thick, echinulate, the

pores 6–8 scattered. Telia epiphyllous, sometimes on sheaths, intercostal, oblong or linear, 0.5–2 mm long, usually numerous, crowded, and often confluent, blackish brown, early naked, ruptured epidermis conspicuous; teliospores obovoid or usually lance-oblong, 21–27 × 42–64 μ, obtuse or usually acute above, somewhat narrowed below, noticeably constricted at the septum; wall chestnut-brown in upper part of spore, lighter below and in apical thickening, 1.5–2 μ thick laterally, 8–13 μ thick apically, smooth; pedicel golden or hyaline, pedicel wall sometimes thickened, up to 65 μ long, persistent.

HOSTS AND DISTRIBUTION: *Spartina gracilis* Trin., *S. pectinata* Link; Canada, U. S. A.

TYPE: Helena, Montana, Sept. 21, 1891, *F. D. Kelsey*, on *Spartina gracilis*, mistaken for *Distichlis spicata* (L.) Greene.

ILLUSTRATIONS: Arth., Bot. Gaz. **34**: 13, f. 3m, n; Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 7, f. 55a; Arth., Man. Rusts, f. 180.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive but they are listed in the original thesis filed in the Purdue University Library.

Puccinia distichlidis is distinguished from the other rusts of Chlorideae by its long, broadly lanceolate, apically thickened teliospores, which are noticeably constricted at the septum. The teliospores are the longest of any of the species on Chlorideae. *Uromyces acuminatus* has been considered by Orton (**18**) to be correlated with this species. There is a close resemblance between the urediospores and acuminately thickened teliospores of both forms.

This species has a distinctly more western range than the other rusts of *Spartina* (*P. seymouriana, P. sparganioides, Uromyces acuminatus*), as only one collection has been recorded from an area east of Iowa. This is a specimen from Cayuga Island, Lasalle, New York on *S. pectinata*. The aecial stage follows a similar range.

12. PUCCINIA KANSENSIS Ell. & Barth., Erythea **4**: 1. 1896 (FIG. 12).

Dicaeoma kansensis (Ell. & Barth.) Arth., Result. Sci. Congr. Bot. Vienne 344. 1906.

Spermogonia and aecia unknown. Uredia usually epiphyllous, scattered, linear or sub-linear, 0.3–0.8 mm long, yellowish, pulverulent, surrounded by ruptured epidermis; urediospores globoid or obovoid, 15–18 × 17–22 μ; wall colorless, 1.5–2 μ thick, echinulate, pores indistinct. Telia usually epiphyllous, scattered, oblong or linear, occasionally con-

fluent, 0.3–0.5 mm long, blackish brown, pulvinate, surrounded by ruptured epidermis; teliospores ellipsoid, rounded at both ends, usually not constricted at the septum, 17–22 × 23–30(–32) μ; wall dark chestnut-brown, 1.5–2.5 μ thick, not or only slightly thicker at apex, smooth, apical and lower pore sometimes depressed; pedicel colorless, thin-walled, collapsing laterally, sometimes up to 30 μ long, persistent or breaking off leaving a short stub attached to teliospores.

HOSTS AND DISTRIBUTION: *Buchloë dactyloides* Engelm.; U. S. A.

TYPE: Rockport, Kan., Sept. 18, 1894, *E. Bartholomew*, on *Buchloë dactyloides*.

ILLUSTRATIONS: Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 9, f. 60a; Syd., Monog. Ured. **1**: pl. 40, f. 524; Arth., Man. Rusts, f. 311.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

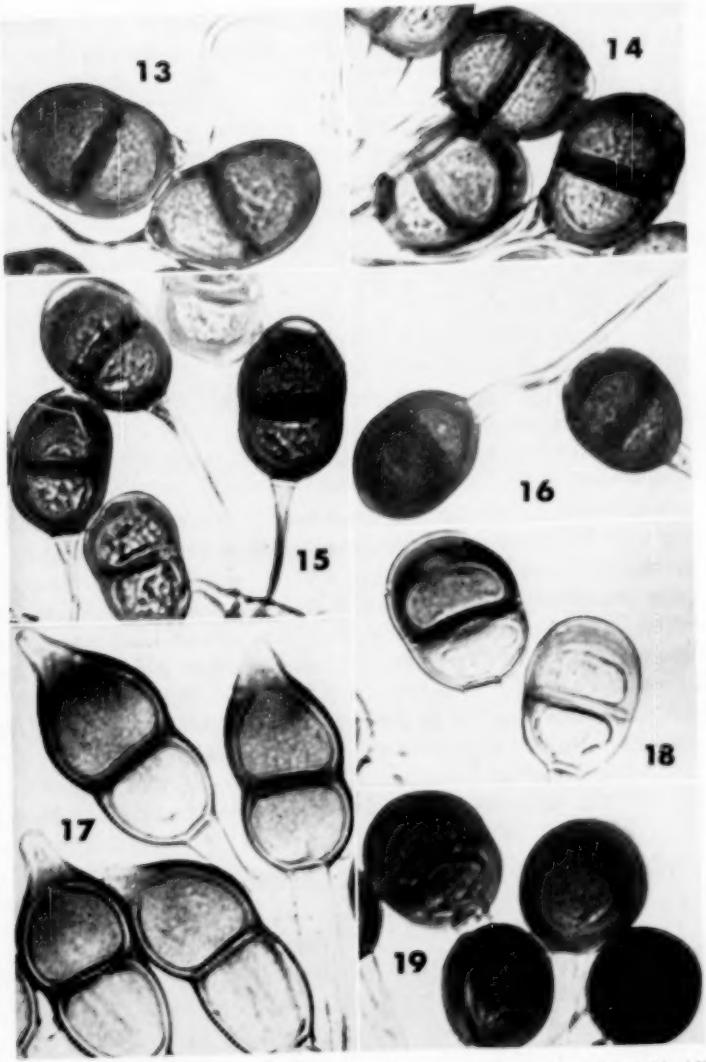
This is an interesting species limited in range to Kansas and Nebraska. Its host is wide-spread over the Great Plains. This limitation may be due to an aecial host with a limited range.

Arthur (**5**) placed this species in the section *Bullaria* of the genus *Puccinia* because the pores of the teliospore are sometimes depressed to a subapical position. The rest of the rusts on Chlorideae are in the section Eupuccinia.

13. PUCCINIA BOUTELOUAE (Jennings) Holw., Ann. Myc. **3**: 20. 1905 (FIG. 14).

Diorchidium boutelouae Jennings, Bull. Texas Exp. Sta. **9**: 25. 1890.
Puccinia gymnopogonis Syd., Monog. Ured. **1**: 755. 1903.
Dicaeoma boutelouae (Jennings) Arth. & Fromme, N. Am. Flora **7**: 319. 1920.
Dicaeoma gymnopogonis (Syd.) Syd., Ann. Myc. **20**: 117. 1922.
Uredo chardonii Kern in Seaver *et al.*, Sci. Surv. Porto Rico and Virgin Is. **8**: 140, N. Y. Acad. Sci. 1932.

Spermogonia and aecia unknown. Uredia amphigenous, elliptical to linear, 0.3–2.0 mm long, orange in the field, becoming colorless in dried specimens, surrounded by the ruptured epidermis; urediospores obovoid, broadly ellipsoid, or sphaeroid, (12–)15–19 × (14–)16–23 μ; wall hyaline to yellowish, (1.5–)2–3 μ thick, strongly echinulate, pores obscure, probably 6–8 scattered. Telia amphigenous and caulicolous, blackish brown, linear, elliptical, oblong or circular, 0.3–3.0 mm long, pulvinate,



FIGS. 13–19. 13. Teliospores of *P. chloridis* (from type of *Puccinia trichloridis*). 14. Teliospores of *Puccinia boutelouae* (from type). 15. Teliospores of *Puccinia chloridis* (from type of *Puccinia bartholomaei*). 16. Teliospores of *Puccinia guaranitica* (from type). 17. Teliospores of *Puccinia cynodontis* (from type). 18. Teliospores of *Puccinia leptochloae* (from type). 19. Teliospores and mesospores of *Puccinia opuntiae* (from type). All × ca. 800.

surrounded by ruptured epidermis; teliospores globoid to broadly ellipsoid, (18–)20–27(–29) × (21–)25–33 μ, rounded at both ends, not constricted at the septum, septum susually vertical; wall chestnut-brown, 2.5–3 μ thick at sides, 5–7 μ thick at apex, smooth; pedicel hyaline to light golden, usually deeper tinted next to spore, up to 100 μ long, thin-walled and usually collapsing laterally, usually attached laterally or at an angle (diorchidioid), persistent.

HOSTS AND DISTRIBUTION: *Bouteloua americana* L. Scribn., *B. curtipendula* (Michx.) Torr., *B. disticha* (H.B.K.) Benth., *B. filiformis* (Fourn.) Griff., *B. heterostega* (Trin.) Griff., *B. juncea* (Desv.) Hitch., *B. rigidiseta* (Steud.) Hitch., *Bouteloua* sp., *Gymnopogon foliosus* (Willd.) Nees; Brazil, British Guiana, Costa Rica, Guatemala, Mexico, Panama, Puerto Rico, Salvador, U. S. A.

TYPE: College Station, Texas, Fall, 1889, *H. S. Jennings*, on *Bouteloua curtipendula*.

ILLUSTRATIONS: Syd., Monog. Ured. **1**: pl. 41, f. 531; Arth., Man. Rusts, f. 186.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive but are listed in the original thesis filed in the Purdue University Library.

An examination of the type of *Puccinia gymnopogonis* proved the rust to be identical to *P. boutelouae*. The diorchidioid form of the teliospores is the only character which separates this species from *P. chloridis*.

Amphispores reported by Arthur (**5**) are urediospores of *P. exasperans* growing on the same leaves with *P. boutelouae*.

14. PUCCINIA CHLORIDIS Speg., Rev. Arg. Hist. Nat. Buenos Aires **1**: 172. 1891 (FIGS. 13, 15).

Aecidium brandegei Pk., Bot. Gaz. **3**: 34. 1878.
Aecidium jamesianum Pk., Bot. Gaz. **5**: 34. 1880.
Puccinia bartholomaei Diet., Hedwigia **31**: 290. 1892.
Dicaeoma bartholomaei (Diet.) Kuntze, Rev. Gen. **3**(3): 468. 1898.
Dicaeoma chloridis (Speg.) Kuntze, Rev. Gen. **3**(3): 468. 1898.
Puccinia jamesiana (Pk.) Arth., Bot. Gaz. **35**: 18. 1903.
Puccinia diplachnis Arth., Bull. Torrey Bot. Club **31**: 4. 1904.
Dicaeoma jamesianum (Pk.) Arth., Result. Sci. Congr. Bot. Vienne 344. 1906.
Dicaeoma diplachnis (Arth.) Arth., Result. Sci. Congr. Bot. Vienne 344. 1906.

Puccinia trichloridis Speg., An. Mus. Nac. Buenos Aires **19**: 298. 1909.

The aecial stage, which occurs on species of *Asclepias, Matelea,* and *Sarcostoma,* has been described by Arthur (**3**, **5**) who has also (**5**) summarized the cultures.

Uredia chiefly epiphyllous, elliptic, 0.5–1.0 mm long, ruptured epidermis evident, pulverulent, pale cinnamon in herbarium specimens, yellow-orange when fresh; urediospores obovoid, broadly ellipsoid, or globoid, 16–22 × 18–23 μ; wall light golden or hyaline, 1.5–2.5 μ thick, usually strongly echinulate, pores obscure, probably 4–8 scattered. Telia chiefly epiphyllous, elliptic to linear, numerous, blackish brown, pulvinate, ruptured epidermis evident; teliospores broadly ellipsoid or oblong, 16–25 × 26–40 μ, usually rounded at both ends or obtuse above and narrowed below, slightly constricted at the septum; wall chestnut-brown, 1.5–2.5 μ thick at sides, 5–9 μ thick at apex, smooth; pedicel light golden-brown, deeper tinted next to spore, up to 100 μ long, wall thinner than lumen, usually collapsing laterally, sometimes collapsing only in lower part, persistent.

HOSTS AND DISTRIBUTION: *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *B. hirsuta* Lag., *Chloris distichophylla* Lag., *Chloris ciliata* Swartz, *Chloris vetusa* Lag., *Chloris* sp., *Leptochloa dubia* (H.B.K.) Nees, *Trichloris mendocino* (Phil.) Kurtz., *T. pluriflora* Fourn.; Argentina, Bolivia, Brazil, Mexico, Paraguay, Uraguay, U. S. A.

TYPE: Paraguari, Paraguay, Feb., 1884, *Balansa,* on *Chloris* sp.

ILLUSTRATIONS: Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 9, f. 59a–e; Arth., Man. Rusts, f. 184.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

The type of teliospores and urediospores present in this species is common to several species which are difficult to separate. This group includes *P. chloridis, P. boutelouae, P. gymnopogonicola,* and *P. dietelii.* The teliospores are variable in size and shape, but generally they are broadly ellipsoid or oblong, and constricted somewhat at the septum. The wall is usually thickened apically and is chestnut-brown. Pedicels are thin-walled, collapse laterally, and are usually more deeply pigmented near the point of attachment. Urediospores are globoid or obovoid, hyaline to light golden, echinulate, and the pores are obscure. It is assumed that there are several (6–8) scattered germ pores.

P. boutelouae differs from *P. chloridis* only in that diorchidioid spores are more frequent than ordinary ones. This is a variable character and

some specimens are difficult to place. Central American and some Southwestern United States collections are predominantly diorchidioid and are therefore placed in *P. boutelouae*. *P. trichloridis* is not distinct. The thick-walled bilamellate teliospore separates *P. gymnopogonicola*. *P. dietelii* has apically thickened urediospores.

15. PUCCINIA SUBTILIPES Speg., Anal. Mus. Nac. Hist. Nat. Buenos Aires **31**: 386. 1922 (FIG. 16).

Spermogonia and aecia unknown. Uredia amphigenous, linear, 0.5–1.5 mm long, surrounded by the ruptured epidermis, pulverulent; urediospores globoid or obovoid, 13–15 × 16–18 μ; wall pale yellow or colorless, 1–1.5 μ thick, echinulate, pores obscure. Telia amphigenous, usually hypophyllous, numerous, rounded or elliptic, blackish brown, 0.3–2 mm long, ruptured epidermis inconspicuous; teliospores short-oblong or broadly ellipsoid, 18–22 × 23–31 μ, rounded at both ends, slightly or not at all constricted at the septum, septum sometimes oblique or vertical; wall evenly dark chestnut-brown, 2–3 μ thick at sides, only slightly thicker at apex, smooth; pedicel light yellow or colorless, light chestnut-brown next to spore, thin-walled and collapsing laterally, up to 130 μ long, persistent.

HOSTS AND DISTRIBUTION: *Leptochloa scabra* Nees, *L. virgata* (L.) Beauv.; Argentina, British West Indies, Costa Rica, Dominican Republic, Guatamala, Honduras, Mexico, Panama, Paraguay.

TYPE: Ascuncion, Paraguay, July, 1919, *C. Spegazzini*, on *Leptochloa virgata*.

ILLUSTRATIONS: Spegazzini, Anal. Mus. Nat. Hist. B. A. **31**: pl. 7, f. 114.

SPECIMENS EXAMINED: Individual specimens are not cited here but are listed in the original thesis, filed in the Purdue University Library.

The small urediospores and long, flexuous teliospore pedicels are the characteristic features of this species.

16. PUCCINIA CYNODONTIS Lacroix ex Desm. in Pl. Crypt. Ser. III, No. *655*. 1859 (FIG. 17).

Aecidium plantaginis Ces., Erb. Critt. Ital. Ser. 1, No. *217*. 1859.
Puccinia cynodontis Fckl., Symb. Myc. Nachr. **2**: 16. 1875.
Aecidium solms-laubachii Boy. & Jacz., Ann. Ecole Nat. d'Agric. Montpellier **1894**: 23. 1894.
Dicaeoma cynodontis (Desm.) Kuntze, Rev. Gen. **3**(3): 468. 1898.
Aecidium marci Bub., Ber. Deutsch. Bot. Ges. **21**: 274. 1903.

Puccinia varians Diet., Ann. Myc. **6**: 224. 1908.
Aecidium raciborskii Wrob., Bull. Sci. Cracovie Classe Sc. Math. Nat. Ser. B. 246. 1916.
Dicaeoma varians (Diet.) Syd., Ann. Myc. **20**: 118. 1922.
Aecidium delphinii-consolidae Hollos, Math. Term. tud. Kozl. **35**: 12. 1926.
Aecidium banaticum Bub. ex J. Jurrisic in Ann. Myc. **34**: 57. 1936.
Uredo elusine-indicae Saw., Jour. Taihoku Soc. Agr. For. **7**: 41. 1943.

The aecial stage, which occurs on species of *Adonis, Delphinium, Viola, Plantago, Valerianella, Mercurialis, Hohenackeria,* and *Veronica,* has been described by Arthur (**3, 5**). A summary of cultures is included below.

Uredia chiefly hypophyllous, scattered or in groups, elliptic to oblong, 0.3–0.6 mm long, pulverulent, cinnamon-brown, surrounded by ruptured epidermis, urediospores globoid, 19–23 × 20–26 μ; wall cinnamon-brown, 2–3 μ thick, verrucose, the pores 2 or 3, equatorial, usually conspicuous. Telia chiefly hypophyllous, crowded, often confluent, elongate on sheaths and culms, oblong-linear, 1–3 mm long, prominent; teliospores oblong or ellipsoid, 16–22 × 28–42 μ, rounded to acuminate or papillate above, obtuse or slightly narrowed below, constricted at the septum, arising from light chestnut-brown basal cells; wall dark chestnut-brown above, paler below and in the narrowed apex, 1.5–2.5 μ thick at sides, 6–12 μ thick at apex, smooth; pedicel uniformly yellow or colorless, thin-walled, sometimes collapsing laterally, up to 75 μ long, persistent.

HOSTS AND DISTRIBUTION: On *Cynodon dactylon* (Pers.) L.; Afghanistan, Algeria, Argentina, Australia, British Guiana, China, Cyprus, Czechoslovakia, France, Formosa, Germany, Guatemala, Hawaii, Honduras, Hungary, India, Italy, Iran, Japan, New Guiana, Puerto Rico, Turkey, U. S. A., U. S. S. R.

TYPE: St. Romain-sur-Vienne, Arroundissement de Chatellerault, en 1857, *De Lacroix,* on *Cynodon dactylon.*

ILLUSTRATIONS: McAlpine, Rusts of Austr., pl. 3, f. 24; Fragoso, Fl. Iber. Uredales **I**: f. 22; Constantineau, Ann. Myc. **14**: 253, f. 5a–u; Doidge, Bothalia **2**: 124; Arthur, Man. Rusts, f. 181; Guyot & Viennot-Bourgin, Ann. Ecole Nat. Agric. Grignon Series III. **3**: 103; Sawada, Jour. Taihoku Soc. **7**: pl. 3, f. 25; Ito, Mycol. Fl. Japan **2**: f. 175; Gutsevich, Surv. Rust Fung. Crimea, f. 6; Savulescu, Mon. Ured. Rep. Pop. Rom., f. 512, 513, 514.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive, but are listed in the original thesis filed in the Purdue University Library.

SUMMARY OF CULTURES: Cultures were made by Tranzschel (**23**) using teliospores from *Cynodon dactylon* on *Plantago* species. Tranzschel, in a letter to J. C. Arthur in 1928, reported successful cultures using teliospores from *Cynodon dactylon* on species of *Adonis, Delphinium, Mercurialis,* and *Veronica.* Bubak (**6**) reported reciprocal cultures between *Cynodon dactylon* and species of *Plantago.* Guyot and Viennot-Bourgin (**13**) successfully used teliospores from *Cynodon dactylon* on *Valerianella cavinata.* Dupais (**10, 11**) used teliospores from *Cynodon dactylon* on species of *Viola, Delphinium, Valerianella, Plantago, Adonis,* and *Mercurialis.*

Puccinia cynodontis has the largest aecial host range known for any rust of the Chlorideae. Members of seven dicotyledonous families have been shown to serve as aecial hosts in Europe and Asia. Uredial and telial stages occur circumglobally on *Cynodon dactylon.*

This was the first rust occurring on the Chlorideae to be described. The first description is meager and contains practically no details about the rust. However, it is certain that the name applies to the rust in question because of the exsiccati which the published description accompanies.

17. PUCCINIA LEPTOCHLOAE Arth. & Fromme, Torreya **15**: 263. 1915.

Dicaeoma leptochloae (Arth. & Fromme) Arth. & Fromme, N. Am. Flora **7**: 321. 1920 (FIG. 18).

Spermogonia and aecia unknown. Uredia hypophyllous, cinnamon-brown, pulverulent, surrounded by ruptured epidermis; urediospores globoid to obovoid, (16–)18–24 × 19–26 μ; wall golden to light cinnamon-brown, 1.5–2.5 μ thick, verrucose, pores 4–6, scattered. Telia usually hypophyllous, sometimes on sheaths and inflorescences, blackish-brown, usually crowded, oval, oblong, or linear, 0.5–2 mm (or more) long, surrounded by ruptured epidermis; teliospores oblong or broadly ellipsoid, 17–24 × 23–26 μ, both ends rounded, slightly constricted at the septum, arising from chestnut-brown basal cells; wall dark chestnut-brown, 2.5–4 μ thick at sides, 4–7 μ thick at apex, smooth; pedicels golden, thick-walled, usually not collapsing laterally, up to 95 μ long, persistent.

HOSTS AND DISTRIBUTION: *Leptochloa filiformis* (Lam.) Beauv.; Argentina, Cuba, Guatamala, Honduras, Mexico, Puerto Rico, U. S. A.

TYPE: Guaymas, Sonora, Mexico, 1887, *E. Palmer,* on *Leptochloa filiformis.*

SPECIMENS EXAMINED: Individual specimens are not cited here but are listed in the original thesis filed in the Purdue University Library.

This species is closely related to *P. opuntiae.* Mesospores are abundant in *P. opuntiae* but they are not abundant in most collections of *P. leptochloae.* However, in two collections, one from Cuba and one from Puerto Rico, they are numerous and two-celled teliospores are rare.

18. PUCCINIA OPUNTIAE Arth. and Holw. in Arthur, Proc. Am. Soc. **64**: 189. 1925 (FIG. 19).

Uredia epiphyllous, elongate, 1–3 mm long, pulverulent, light cinnamon-brown, ruptured epidermis conspicuous; urediospores globoid or broadly ellipsoid, 19–26 × 20–26 μ; wall light cinnamon-brown or golden, 2–2.5 μ, verrucose, the pores usually 6 sometimes 8, scattered, sometimes bizonate, distinct. Telia amphigenous, oblong or linear 0.5–4 mm long, early naked, pulvinate, prominent, blackish brown, ruptured epidermis not apparent; teliospores broadly ellipsoid 19–26 × 26–45 μ, rounded at both ends, or somewhat narrowed below, not or slightly constricted at the septum; wall dark chestnut-brown, 2–2.5 μ thick laterally, 4–9 μ thick apically, smooth; pedicel pale yellow or hyaline, up to 80 μ long, thick-walled, not collapsing; mesospores very numerous, globoid to broadly ellipsoid or obovoid, 21–25 × 24–28 μ; wall dark chestnut-brown, 3–4 μ thick at sides, 5–9 μ thick at apex; pedicel thick-walled, light golden, up to 70 μ long, persistent.

HOST AND DISTRIBUTION: On *Bouteloua simplex* Lag.; Bolivia, Peru.

TYPE: Cochabamba, Bolivia, Mar. 2, 1920, *E. W. D. & M. M. Holway* (Reliquiae Holwayanae, No. *56*), on *Bouteloua simplex.*

SPECIMENS EXAMINED: On *Bouteloua simplex:* BOLIVIA: Cochabamba, Mar. 2, 1920, *Holway* (Reliq. Holw. No. *56;* TYPE); Cochabamba, Cerro San Pedro, Mar., 1947, *Cardenas 3923;* PERU: Dept. de Lima, Apr. 4, 1946, *Ferreyra 649.*

When naming this species, Arthur and Holway assumed that the aecial connection was *Aecidium opuntiae* Magn. Although this aecial connection is probably correct, no proof by culture work has shown it to be so.

The relation of this species to *P. leptochloae* is discussed under the latter.

KEY TO SPECIES OF UROMYCES

1. Urediospores with 3–4 equatorial pores.
   2. Teliospores smooth.
      3. Telia erumpent.
         4. Urediospores echinulate, teliospores with thin-walled pedicels.
            5. Teliospores with apical wall thickened 7–10 μ, urediospore wall 2–3 μ thick (*Spartina*) ..... 1. *U. argutus.*
            5. Teliospores with apical wall thickened 3–6 μ, urediospore wall 1–1.5 μ thick (*Astrebla, Trichoneura, Tripogon*) ..... 2. *U. trichoneurae.*
         4. Urediospores verrucose, teliospores with thick-walled pedicels (*Chloris, Leptochloa*) ..... 3. *U. archerianus.*
      3. Telia covered by epidermis.
         6. Teliospores with apical wall thickened 4–5 μ, urediospores cinnamon brown (*Dactyloctenium*) ..... 4. *U. dactyloctenii.*
         6. Teliospores with apical wall thickened 2.5–3 μ, urediospores light yellow (*Microchloa*) ..... 5. *U. microchloae.*
   2. Teliospores tuberculate (*Dactyloctenium*) ..... 6. *U. dactylocteniicola.*
1. Urediospores with 6–10 scattered pores.
   7. Teliospores with pedicels up to 60 μ long, urediospores 15–20 × 17–24 μ (*Chloris*) ..... 7. *U. kenyensis.*
   7. Teliospores with pedicels up to 35 μ long, urediospores 19–26 × 23–29 μ.
      8. Apical wall of teliospore variable, rounded, truncate, acuminate, or papillate, side wall 1–1.5 μ thick (*Spartina*) ..... 8. *U. acuminatus.*
      8. Apical wall of teliospore rounded, side wall 2–3.5 μ thick (*Leptochloa*) ..... 9. *U. leptochloae.*

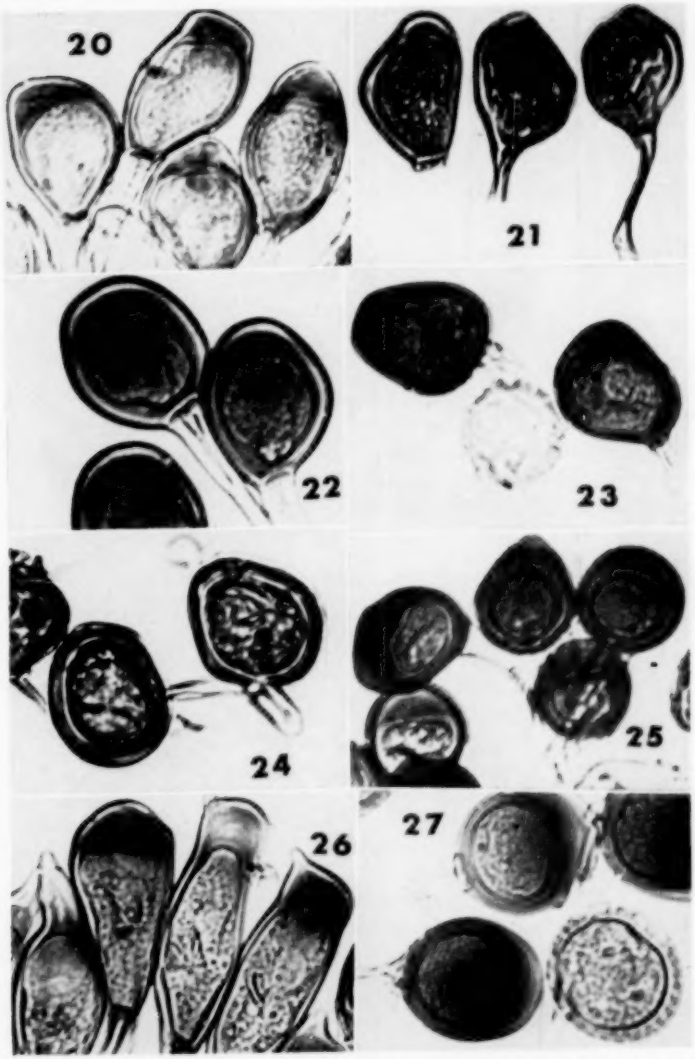
DESCRIPTIONS OF SPECIES

1. UROMYCES ARGUTUS Kern, Torreya **11**: 214. 1911 (FIG. 20).

Uredo spartinae-strictae Pat. & Har., Bull. Soc. Mycol. Fr. **21**: 84. 1905.

Nigredo argutus (Kern) Arth., N. Am. Flora **7**: 232. 1912.

Spermogonia and aecia unknown. Uredia amphigenous, scattered, associated with rather large brownish or purplish spots and usually solitary in American specimens, pulverulent, cinnamon-brown, tardily naked, surrounded by ruptured epidermis; urediospores oblong or broadly ellipsoid, 18–24 × 25–35 μ; wall 2–3 μ thick, golden to clear chestnut, echinulate, pores 3 or 4, approximately equatorial, usually covered with hyaline umbos. Telia amphigenous, scattered, sometimes associated with discolored spots, linear, 1–2 mm long, tardily naked, surrounded by ruptured epidermis, blackish brown; teliospores ellipsoid, usually narrowed both above and below, or truncate above, 15–19 × 24–39 μ; wall clear chestnut-brown, 1.5–2 μ thick at sides, 7–10 μ thick at apex, smooth; pedicel golden, up to 70 μ long, thin-walled and collapsing laterally, persistent.



FIGS. 20–27. 20. Teliospores of *Uromyces argutus* (from type). 21. Teliospores of *Uromyces trichoneurae* (from type). 22. Teliospores of *Uromyces archerianus* (from type). 23. Teliospores and urediospore of *Uromyces dactyloctenii* (from type). 24. Teliospores of *Uromyces microchloae* (from type). 25. Teliospores of *Uromyces kenyensis* (from type). 26. Teliospores of *Uromyces acuminatus* (from type). 27. Teliospores and urediospore of *Uromyces leptochloae* (from type). All × ca. 800.

HOSTS AND DISTRIBUTION: *Spartina alterniflora* Loisel; France, U. S. A.

TYPE: Miami, Florida, March 25, 1903, *E. W. D. Holway*, on *Spartina alterniflora.*

SPECIMENS EXAMINED: On *Spartina alterniflora,* FRANCE: Le Croisic, *Menier* (FH), type of *Uredo spartinae-strictae;* U. S. A.: Miami, Fla., Mar. 25, 1903, *Rolfs;* Mar. 25, 1903, *Holway* (TYPE); May 15, 1906, *Bessey.*

Uredo spartinae-strictae is known from two recorded collections from the type locality in France. Examination of the type proved it to be *Uromyces argutus.*

The teliospores resemble those of coastal collections of *Uromyces acuminatus,* making it necessary to have urediospores in order to establish certain identity. The three equatorial pores of the urediospores separate this species from *U. acuminatus,* while its lack of apically thickened urediospores distinguishes the *Uredo* stage from *Puccinia sparganioides,* occurring also on *Spartina.* This is the only rust recorded outside of North and South America on *Spartina.*

2. UROMYCES TRICHONEURAE Doidge, Bothalia **3**: 512. 1939 (FIG. 21).

Uromyces tripogonicola Payak & Thirum. (*nom. nud.*) in Payak, Sci. and Cult. **18**: 324–343. 1953.

Spermogonia and aecia unknown. Uredia amphigenous, scattered, small, pulverulent, tardily naked, surrounded by ruptured epidermis; urediospores subgloboid to ellipsoid or ovoid, 19–26 × 24–33 μ; wall clear cinnamon-brown, 1.5–2 μ thick, echinulate, pores 3 to 6, equatorial or, on *Astrebla,* variable. Telia epiphyllous, dark chocolate-brown, tardily naked, surrounded by the ruptured epidermis; teliospores subglobose, ovate, oblong or pyriform, usually angulate, rounded or truncate at apex, rounded or attenuate below, 15–24 × 21–31 μ; wall chestnut-brown, 2 μ thick at sides, 3–7 μ thick at apex, smooth; pedicel thin-walled, yellowish, collapsing laterally, up to 45 μ long, persistent.

HOSTS AND DISTRIBUTION: *Astrebla elymoides* F. Muell., *A. lappacea* (Lindl.) Domin, *Trichoneura grandiglumis* (Nees) Ekmann, *Tripogon lisboae* Stapf.; Transvaal, India, Australia.

TYPE: Donkerpoort, Pretoria, Transvaal, S. Africa, May 4, 1937, *Doidge & Bottomley* No. *29762,* on *Trichoneura grandiglumis.*

ILLUSTRATIONS: Doidge, Bothalia **3**: 512, f. 37.

SPECIMENS EXAMINED: On *Astrebla elymoides,* AUSTRALIA: Lawes, Queensland, June 10, 1940, *Langdon 78;* on *A. lappacea,* AUSTRALIA:

Dalby, Queensland, May 27, 1941, *Langdon 278*, Lawes, Queensland, June 10, 1940, *Langdon 74, 75;* on *Trichoneura grandiglumis*, TRANSVAAL: Donkerpoort, Pretoria dist., May 4, 1937, *Doidge & Bottomley 29762* (TYPE); on *Tripogon lisboae*, INDIA: Purandhar Hill Fort, Poona distr., Bombay state, Oct. 22, 1950, *Payak* (co-type of *Uromyces tripogonicola*).

The telia and teliospores on *Astrebla*, *Tripogon*, and *Trichoneura* are identical but the urediospores are variable. Urediospores from *Astrebla* have 4 to 6 pores variable in position but mostly in the equatorial zone, those from *Tripogon* have 4–6 (rarely 3) in the equator, while those from the type on *Trichoneura* have 3 or 4 in the equator. Spores from the type are slightly larger and are usually slightly darker. With the limited collections now available we do not believe that the variations justify recognition of more than one species.

3. UROMYCES ARCHERIANUS Arth. & Fromme, Torreya **15**: 261. 1915 (FIG. 22).

Uromyces chloridis Doidge, Bothalia **2**: 46. 1927.
Nigredo archerianus (Arth. and Fromme) Arth., N. Am. Flora **7**: 747. 1926.

Spermogonia and aecia unknown. Uredia hypophyllous, minute, linear, light brown, tardily naked; urediospores globoid or broadly ellipsoid, 18–26 × 21–29 μ; wall golden to cinnamon-brown, 2–3 μ thick, verrucose, pores 2 or 3, equatorial. Telia amphigenous, mostly hypophyllous, prominent, blackish brown, oblong or linear, 0.5–2 mm long, often confluent and forming pulvinate streaks up to 10 mm long and 1.5 mm broad; teliospores globoid or occasionally broadly ellipsoid, rounded above and below, or subattenuate at the base, 17–25 × 23–33 μ; wall chestnut-brown, 2–3 μ thick at sides, 7–9 μ thick at apex, smooth; pedicel hyaline to yellowish, thick-walled, not collapsing, up to 120 μ long, persistent.

HOSTS AND DISTRIBUTION: *Chloris breviseta* Benth., *Chloris virgata* Sw., *Enteropogon monostachyus* (Vahl) Schum.; Tanganyika, Uganda, Union of S. Africa, U. S. A.

TYPE: Mesilla Park, N. Mex., U. S. A., Nov. 12, 1914, *W. A. Archer*, on *Chloris virgata*.

SPECIMENS EXAMINED: On *Chloris breviseta*, UGANDA: Kampala, Feb., 1930, *Hansford 1040;* on *C. virgata*, UNION OF SOUTH AFRICA: Bloemfontein, Orange Free State, Apr., 1917, *Potts* (TYPE of *Uromyces chloridis* Doidge); U. S. A.: Mesilla Park, N. Mex., Nov. 12, 1914,

Archer (TYPE); Feb. 7, 1915, *Archer;* Mar. 22, 1915, *Archer;* on *Enteropogon monostachyus,* TANGANYIKA: Moshi, Sept. 1, 1929, *Hitchcock 24580.*

No differences could be found between *Uromyces chloridis* Doidge and *U. archerianus* Arth. and Fromme.

In America this species has been found only at Mesila Park, New Mexico, and only during one season. Subsequent efforts to collect the rust have failed. The rust is probably endemic to Africa and its occurrence for a season in North America must be explained on chance importation.

The collection on *Enteropogon monostachyus* differs from the collections on *Chloris* in that teliospores measure 16–23 × 20–24 μ. This difference is not considered significant enough for designating a subspecific taxon.

This species can be identified by the verrucose urediospores with equatorial pores and the globoid teliospores with thick-walled noncollapsing pedicels.

4. UROMYCES DACTYLOCTENII Wakef. & Hansf., Proc. Linn. Soc. London **161**: 170. 1949 (FIG. 23).

Spermogonia and aecia unknown. Uredia amphigenous, cinnamon-brown, blister-like, epidermis rupturing tardily and surrounding uredia afterwards, oblong or broadly ellipsoid, 1–2 mm long, pulverulent; urediospores globoid to broadly ellipsoid, 18–24 × 22–27 μ; wall light golden to light cinnamon-brown, 1.5–2.5 μ thick, echinulate, pores 3 or 4, equatorial. Telia amphigenous, intermixed with the uredia, covered by the epidermis, forming irregular blackish spots 0.2–0.4 mm in diameter; teliospores irregular, globoid, ovoid, or pyriform, usually angulate, fragile, 18–24 × 23–30 μ; wall chestnut-brown, 1–2 μ thick at sides, the apex 4–5 μ thick, smooth; pedicel hyaline or yellowish, thin-walled, fragile, collapsing laterally, up to 15 μ long, usually broken off shorter.

HOSTS AND DISTRIBUTION: *Dactyloctenium aegypticum* (L.) Beauv.; Kenya, Uganda.

TYPE: Katakwi, Teso, Uganda, Aug., 1933, *C. G. Hansford* No. *1653,* on *Dactyloctenium aegypticum.*

ILLUSTRATION: Wakefield and Hansford, Proc. Linn. Soc. London **131**: 170, f. 7.

SPECIMENS EXAMINED: On *Dactyloctenium aegypticum,* KENYA: Nairobi, Apr., 1950, *Nattrass 1411;* UGANDA: Mamanyni (sp.?), Apr., 1916, *Dummer 2795;* Bugishu, Budama, Bunyuli, June, 1927, *Snowden 1128;* Katakwi, Teso, Aug., 1933, *Hansford 1653* (TYPE).

This species is closely related to *U. microchloae*. The two species differ from other rusts on *Chlorideae* in that they have covered telia (except for the questionable species, *U. dactyloctenii*icola, reported to have covered telia).

The uredial paraphyses, reported in the original description of *U. dactyloctenii*, are urediospore pedicels.

5. UROMYCES MICROCHLOAE Syd., Ann. Myc. **1**: 15. 1903 (FIG. 24).

Spermogonia and aecia unknown. Uredia amphigenous, small, scattered, tardily naked; urediospores broadly ellipsoid to globose, 16–24 × 18–28 μ; wall hyaline to light golden, echinulate, 1.5–2 μ thick. Telia amphigenous, scattered, blackish brown, covered by the epidermis; teliospores globoid to broadly ellipsoid, rounded above and below, sometimes angular, 18–25 × 20–29 μ; wall deep golden to clear chestnut-brown, 2–3.5 μ thick, smooth; pedicel thin-walled, collapsing laterally, hyaline, up to 25 μ long, persistent.

HOSTS AND DISTRIBUTION: *Microchloa indica* (L.) Beauv., *M. setacea* R. Br.; Bolivia, Brazil, Central Africa.

TYPE: Seriba Ghattas, Central Africa, Sept. 12, 1869, *G. Sweinfurth*, on *Michochloa setacea*.

ILLUSTRATIONS: Syd., Monog. Ured. **2**: pl. 13, f. 165.

SPECIMENS EXAMINED: On *Microchloa indica*, BOLIVIA: Cochabamba, Mar. 3, 1920, *Holway 361* (Reliq. Holw. No. 57); La Paz, Mar. 31, 1920, *Holway 490* (Reliq. Holw. No. 74); BRAZIL: locality and date not given, *Burchell;* on *M. setacea*, CENTRAL AFRICA: Seriba Ghattas, Sept. 12, 1869, *Sweinfurth* (TYPE).

Although the Sydows described urediospores, they are not present in the portion of the type in the Arthur Herbarium. The urediospore description given here is from the South American collections. Since urediospores have not been seen from the type there may be some question as to whether the African and South American collections are the same rust.

Collections of this species from Bolivia show two size ranges for urediospores. The urediospores from Cochabamba are 16–18 × 18–24 μ, while those from La Pas are 21–24 × 25–28 μ.

The relation to *U. dactyloctenii* is discussed under the latter.

6. UROMYCES DACTYLOCTENIICOLA (Speg.) Lindquist, Notas Museo La Plata **8**: 136. 1943.

*Uredo dactyloctenii*icola Speg., Anal. Mus. Nac. Hist. Nac. Buenos Aires **31**: 40. 1922.

Spermogonia and aecia unknown. Uredia small, yellowish, amphigenous, pulverulent; urediospores oblong or globose, 18–22(–26) × 18–22(–25) μ; wall golden-yellow, echinulate, 2–3 μ thick, with 3 equatorial germ pores; telia small, up to 5 mm long, black, covered by the epidermis; teliospores oblong or ellipsoid, asymmetrical, 18 × 22–25 μ, usually with a small hyaline umbo over the apical pore, wall cinnamon-brown, coarsely verrucose; pedicel hyaline, short and fragile.

HOSTS AND DISTRIBUTION: *Dactyloctenium aegypticum* (L.) Beauv.; Paraguay.

TYPE: Ascuncion, Paraguay, July, 1919, *Spegazzini*, on *Dactyloctenium aegypticum*.

ILLUSTRATIONS: Lindquist, Notas Museo La Plata **8**: 136.

SPECIMENS EXAMINED: Type only.

The description given here is taken from Lindquist.

The status of this species is uncertain. Spegazzini reported in his observations on *Uredo dactyloctenii* that he saw spores which might be teliospores. Lindquist made a restudy of the type, stated that warted teliospores were present and, therefore, transferred it to *Uromyces*. We have examined the type and have found verrucose teliospores but it is not certain that they were growing on the grass. They closely resemble species of *Uromyces* occurring upon the *Euphorbiaceae* and may possibly have come from such a source.

7. **Uromyces kenyensis** Hennen, sp. nov. (FIG. 25).

Spermogoniis et aecidiis ignotis. Urediis hypophyllis, linearibus, confluentibus, usque 3 mm longis, pulverulentis, cinnamoneo-brunneis; urediosporae globoideis, 15–20 × 17–24 μ; membrana cinnamoneo-brunnea, 1.5–2.5 μ crassa, echinulata, poris germ. 6–8, sparsis. Teliis hypophyllis, castaneo-brunneis, pulverulentis; teliosporis globoideis vel late ellipsoideis, 17–20 × 18–24 μ; membrana castaneo-brunnea, 2–2.5 μ crassa, ad apicem 5–8 μ crassa, levi; pedicello hyalino vel flavido, usque 60 μ longo, persistenti.

Spermogonia and aecia unknown. Uredia amphigenous, chiefly hypophylous, linear, confluent, up to 3 mm long, pulverulent, cinnamon-brown, surrounded by the ruptured epidermis; urediospores globoid, 15–20 × 17–24 μ, wall cinnamon-brown, 1.5–2.5 μ thick, echinulate; pores 6–8, scattered or bizonate. Telia hypophyllous, chestnut-brown, pulverulent, surrounded by ruptured epidermis; teliospores globoid or broadly ellipsoid, 17–20 × 18–24 μ; wall chestnut-brown, 2–2.5 μ thick at sides, 5–8 μ thick at apex, smooth; pedicel thin-walled, collapsing laterally, hyaline or yellowish, up to 60 μ long, persistent.

HOSTS AND DISTRIBUTION: On *Chloris myriostachya* Hochst.; Nairobi, Kenya.

TYPE: Nairobi, Kenya, May, 1950, *R. M. Nattrass* No. *1427*, on *Chloris myriostachya*.

SPECIMENS EXAMINED: Type only.

This species is distinguished from other *Uromyces* on Chlorideae which have scattered or bizonate pores in the urediospores by its longer teliospore pedicel, and smaller urediospores and teliospores.

8. UROMYCES ACUMINATUS Arth., Bull. Minn. Acad. **2**: 35. 1883 (FIG. 26).

Aecidium polemonii Pk., Bot. Gaz. **4**: 230. 1879.
Uromyces spartinae Farl., Proc. Am. Acad. **18**: 77. 1883.
Aecidium tissae Ell. & Ev., Bull. Torrey Bot. Club **24**: 284. 1897.
Caeomurus acuminatus (Arth.) Kuntze, Rev. Gen. **3**(3): 450. 1898.
Caeomurus spartinae (Farl.) Kuntze, Rev. Gen. **3**(3): 450. 1898.
Aecidium magnatum Arth., Bull. Torrey Bot. Club **28**: 664. 1901.
Nigredo acuminata (Arth.) Arth., Result. Sci. Congr. Bot. Vienne 343. 1906.
Nigredo polemonii (Pk.) Arth., N. Am. Flora **7**: 231. 1912.
Uromyces magnatus (Arth.) Arth., Mycologia **9**: 311. 1917.
Uromyces acuminatus magnatus (Arth.) J. J. Davis, Trans. Wis. Acad. Sci. **1922**: 410. 1923.
Uromyces acuminatus polemonii (Pk.) J. J. Davis, Trans. Wis. Acad. Sci. **1922**: 411. 1923.

The aecial stage, which occurs on species of *Arenaria, Spergularia, Maianthemum, Polygonatum, Smilacina, Uvularia, Dodecatheon, Lysimachia, Steironema, Collomia, Gilia, Phlox*, and *Polemonium*, has been described by Arthur (**3**, **5**) who has also (**5**) summarized the cultures.

Uredia epiphyllous, scattered, intercostal, linear, 0.4–2 mm or more long, cinnamon-brown, ruptured epidermis conspicuous; urediospores globoid, 19–30 × 23–39 μ; wall 2–3.5 μ thick, yellow to golden, echinulate, pores 8, scattered. Telia epiphyllous, scattered, intercostal, linear, 0.4–2 mm or more long, blackish brown, surrounded by ruptured epidermis, teliospores oblong-obovoid or ellipsoid, rounded, obtuse, acuminate or papillate above, usually narrowed below, 15–24 × 23–39 μ; wall chestnut-brown, sometimes with a paler papilla or beak, often paler below, 1–1.5 μ thick at sides, 5–12 μ at apex, smooth; pedicel light yellow, thin-walled and collapsing laterally, up to 25 μ long, persistent.

HOSTS AND DISTRIBUTION: *Spartina alterniflora* Loisel, *S. cynosuroides* (L.) Roth, *S. gracilis* Trin., *S. pectinata* Link, *S. patens* (Ait.) Muhl., *Spartina* sp.; Canada, U. S. A.

TYPE: Ft. Dodge, Iowa, Sept., 1882, *J. C. Arthur*, on *Spartina pectinata*.

ILLUSTRATIONS: Arth., Bot. Gaz. **34**: 5, f. 1a, b, c, d, e; Arth. & Holw., Bull. Lab. Nat. Hist. Univ. Iowa **5**: pl. 4, f. 52a, b, c, d; Syd., Monog. Ured. **2**: pl. 14, f. 524.

SPECIMENS EXAMINED: Individual specimens are not cited here since the records are extensive but they are listed in the original thesis filed in the Purdue University Library.

This is a species complex exhibiting considerable diversity. The aecial host range includes four families and is second only to *Puccinia cynodontis* with seven families. *U. acuminatus* is unique in that both monocotyledonous and dicotyledonous families may serve as aecial hosts.

The telial stage may be divided into two intergrading morphological groups. All collections from the Atlantic coastal region have rounded, truncate or broadly acuminate teliospores. Some collections from the northern Great Plains and Colorado are also like this. The other inland collections are variable and at least some of the teliospores are sharply acuminate or papillate apically. These two groups are not supported by differences in urediospores or aeciospores.

9. UROMYCES LEPTOCHLOAE Wakef. in Wakefield & Hansford, Proc. Linn. Soc. London **161**: 172. 1949 (FIG. 27).

Spermogonia and aecia unknown. Uredia not seen, urediospores mixed with teliospores; urediospores globose to broadly ellipsoid, 20–26 × 24–28 μ; wall pale to deep cinnamon-brown, 2.5–3.5 μ thick, echinulate, pores 8–10, scattered. Telia broadly ellipsoid to linear, blackish brown, 0.5–1 mm long, tardily naked; teliospores globose to ovate, rounded below and above, 20–24 × 22–27 μ; wall clear chestnut-brown, somewhat lighter apically, 2–3.5 μ thick at the sides, 7–10 μ thick apically, smooth; pedicel thin-walled, hyaline, collapsing laterally, up to 35 μ long, persistent.

HOSTS AND DISTRIBUTION: *Leptochloa obtusiflora* Hochst.; Uganda.

TYPE: Tororo, Uganda, Jan. 1929, *C. G. Hansford*, on *Leptochloa obtusiflora*.

ILLUSTRATIONS: Wakef., Proc. Linn. Soc. London **161**: 172, f. 8.

SPECIMENS EXAMINED: On *Leptochloa obtusiflora*, UGANDA: Bugondo Hills, Teso, Mar., 1933 (UGAN); Tororo, Jan., 1929, *Hansford* (TYPE) (UGAN).

EXCLUDED AND DOUBTFUL SPECIES

1. PUCCINIA CAMPULOSAE Thuem., Bull. Torr. Bot. Club **6**: 215. 1878.

Dicacoma campulosi (Thuem.) Kuntze, Rev. Gen. **3**(3): 468. 1898.

Arthur (**3**) reports that the specimen on which this species is based was destroyed by fire, and that the description given by von Thuemen is too meager to fully establish the species as wholly distinct. Arthur tried unsuccessfully to find additional material in the phanerogamic collections at the New York Botanical Garden, Academy of Sciences at Philadelphia, National Herbarium at Washington, and the Field Museum at Chicago. Likewise, we have been unable to find rust on *Ctenium* species in the National Herbarium at Washington, Chicago Museum of Natural History, or the Missouri Botanical Garden.

2. PUCCINIA BUCHLOES Schofield ex Webber in Ann. Rep. Neb. Board Agr. **1889**: 68. 1890.

Puccinia buchloes (Webb.) Syd., Monog. Ured. **1**: 740. 1904.

This name was never published as such. It was only mentioned in the discussion under a rust labeled as *Puccinia* sp. Subsequently this rust was found to be *P. aristidae* Tracy on *Distichlis spicata*.

3. PUCCINIA DESMAZIERESI Constan., Ann. Myc. **14**: 251–253. 1916.

The type specimen of this species was examined. The leaves were found to be unlike *Cynodon dactylon* on which it was reported. They were sent to Agnes Chase who identified the grass as probably *Cleistogenes serotina* (L.) Keng. The rust is probably *Puccinia moliniae* Tul.

4. UREDO BOUTELOUAE Arth., Bull. Iowa Agr. Coll. Dept. Bot. **1884**: 164. 1884.

This *Uredo* was reported on *Bouteloua curtipendula*. It was later found to be *Puccinia poae-nemoralis* Otth. (*P. poae-sudeticae* (West.) Jorst.) on *Poa pratensis*.

5. UREDO CTENII Wakef. & Hansf. (*nomen nudum*) in Hansford, East African Agric. Journ. **3**: 319–324. 1938.

This *Uredo* was reported on an unknown species of *Ctenium* but no description has been published.

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THE OCCURRENCE OF ARACHNIOTUS CITRINUS IN SOILS

BILLY WEST [1] AND LIBERO AJELLO [2]

(WITH 1 FIGURE)

Arachniotus citrinus was first described by Massee and Salmon in 1902 (3). This member of the family Gymnoascaceae was observed growing on kangaroo dung collected in the London Zoo. Morphological studies were made on the gross specimen only, as cultivation of the fungus on synthetic media was not attempted. Since that date no further observations of this ascomycete have been reported.

Recently, while a large number of Tennessee soil samples were being screened for the presence of *Histoplasma capsulatum* and *Microsporum gypseum*, two soil samples yielded *A. citrinus*. One isolate was recovered indirectly from soil by passage through mice (2), while the second was observed developing adventitiously on strands of sterilized human hair used as bait for isolation of *M. gypseum* (1). *A. citrinus* grew readily on Sabouraud dextrose agar, forming a white cottony colony which slowly turned lemon-yellow [3] in color as asci and ascospores were developed. On Czapek's agar mycelial development was sparse but numerous lemon-yellow clusters of asci were formed. There was scant production of mycelium on sterilized rabbit dung pellets and rabbit dung extract agar (FIG. 1A). However, this medium was excellent for inducing ascus formation.

Microscopic examination of the fungus grown on all these media revealed essentially similar findings. It was noted that copulation branches arose from mycelial filaments and coiled about each other (FIG. 1B). From these copulation branches asci were successively developed (FIG. 1C). This successive development of asci from the copulation branches led to the formation of clusters of asci along the hyphae (FIG. 1D). No signs of a peridium were found. Each ascus enclosed eight elliptical, smooth, lemon-yellow spores (FIG. 1D). When mounted in

[1] 1st Lt. USAF (MSC).

[2] Communicable Disease Center, Public Health Service, United States Department of Health, Education and Welfare, Atlanta, Georgia.

[3] Munsell Color Standard (4) formula 7.5Y 9/10; close to Ridgeway's (5) lemon-yellow.

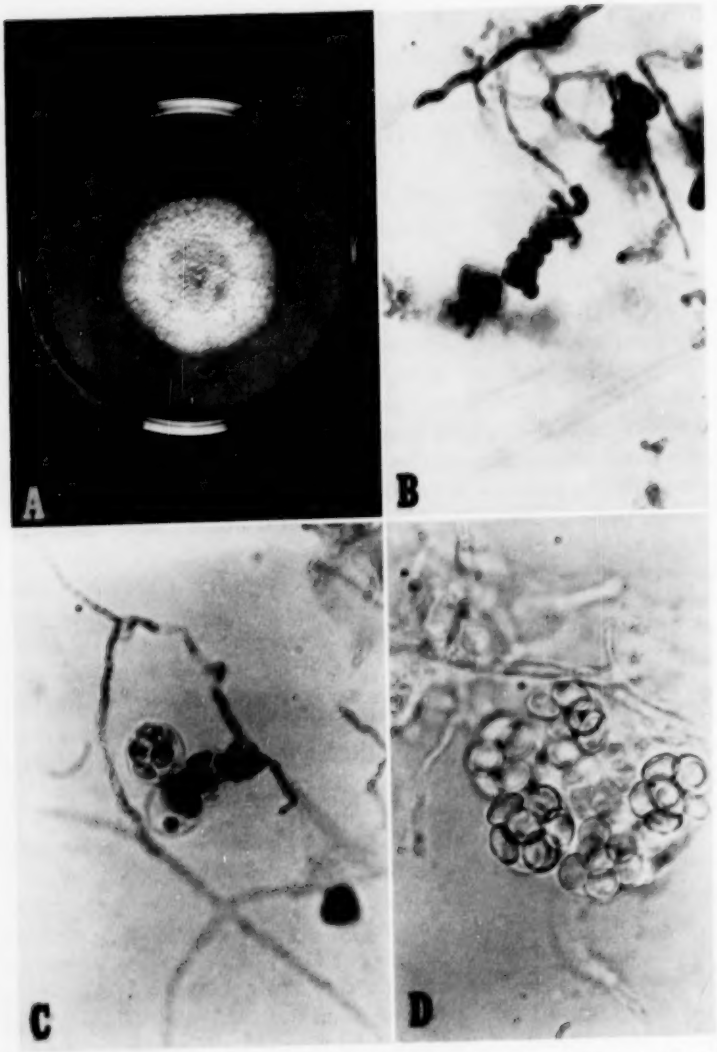

FIG. 1. *Arachniotus citrinus*. A. Three-week-old colony growing on rabbit dung extract agar. B. Coiled copulation branches, × 400 C. Asci arising from copulation branches, × 655. D. Cluster of asci containing smooth walled, elliptical ascospores, × 800.

lactophenol cotton blue, the asci measured 10.9–12.7 μ and the ascospores 4.7 × 6.0 μ. These measurements differ from those cited by Massee and Salmon. The asci of their isolate measured 8–10 μ in diameter and the ascospores ranged in size from 2.5–3.5 μ by 4.0–5.0 μ. However, we do not consider these size differences of great enough magnitude to be taxonomically significant. This impression was confirmed by examination of Massee's original drawings of *A. citrinus*.[4]

Subcultures of one of the Tennessee isolates of *A. citrinus* have been deposited in the culture collection of the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, and the American Type Culture Collection, Washington, D. C.

SUMMARY

1. *Arachniotus citrinus* was recovered from one Tennessee soil sample and observed on another by methods used to isolate *Histoplasma capsulatum* and *Microsporum gypseum*.

2. The morphological features of this ascomycete are described and illustrated.

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[4] Obtained through the courtesy of Dr. D. P. Rogers of the New York Botanical Garden.

NOTES AND BRIEF ARTICLES

Growth of the yeast phase of Histoplasma capsulatum in a Simplified Fluid Medium

To date, no liquid media of simple composition have been described that can furnish extensive yeast-like growth of *Histoplasma capsulatum*. The medium reported below has the advantage of producing a rich harvest in a reasonable incubation time without the need of agitation or the addition of whole blood. Further, the clear and transparent medium favors turbidometric readings.

Formula per Liter of Distilled Water

Glucose	80 g
Ammonium nitrate	350 mg
Magnesium sulfate	80 mg
Iron sulfate	5 mg
Zinc sulfate	5 mg
Potassium diphosphate	80 mg
Ox serum	10 ml

With the exception of glucose and serum, all substances are dissolved in water: the liquid medium is adjusted to pH 6.5 and sterilized at 15 lb (120° C). Sterile glucose solution and serum are added prior to use. To date, no substitute for serum has been found. Additions of p-aminobenzoic acid, folic, glutamic, and aspartic acids did not serve to replace serum.

In this medium, inocula necessary for good growth may be made using less than 50,000 cells/ml. As with more complex media, an incubation temperature of 37° C is essential if the dimorphism phenomenon is to be averted.

Stock cultures of *Histoplasma capsulatum* in the yeast phase may be maintained on agar slants prepared with this medium. A monthly transfer is recommended. In order to insure sufficient moisture in the solid media during incubation, two techniques are suggested: the slants may be sealed to prevent dissemination of moisture and incubated in a horizontal position; or the screw caps may be loosely adjusted, provided the incubator is supplied with the necessary humidity.—Roland Bonorden, Research Department, CIBA Pharmaceutical Products Inc., Summit, New Jersey.

THREE RHODE ISLAND POLYPORES

In June, 1955, three fruiting bodies of *Polyporus sulphureus* Bull. ex Fr. were found about 6 feet from the ground on a *Cladrastis lutea* of 2 feet DBH in Providence. At the level of the fruit bodies, the heartwood was completely decayed and it appeared that the decay of the trunk below the branches was quite extensive. This host is not listed for *P. sulphureus* in Overholts' recent treatise of the polypores and J. L. Lowe writes that he has not known of it before.

Var. *semialbinus* Pk. of *P. sulphureus* is recorded by Overholts as known from Pennsylvania, Ohio and Arkansas, although the statement is added that the distribution is probably as wide as that of the species. Good-sized fruit bodies, with well-developed sclerotia, were found four times in Providence this past year— on June 22nd and July 29th in the same spot at the base of a very large *Quercus velutina*, and on August 16th on the opposite side of the same tree, and on August 5th on the ground 6–8 feet from a *Quercus alba*. Cultures were made of two of these collections.

A second polypore collected for the first time in Rhode Island as far as is known was *Polyporus griseus* Pk.—under hemlocks with an oak and a white pine 10 to 15 feet away, near the Connecticut line in the town of Gloucester. This species is found in either coniferous or deciduous woods but it may be of some interest to note incidentally that Henry A. C. Jackson not uncommonly finds it in his section of L'Islet County in the Province of Quebec growing in stands of pure *Thuja occidentalis*.—WALTER H. SNELL AND ESTHER A. DICK, Brown University, Providence 12, Rhode Island.

A CONTRIBUTION TO THE FUNGI OF EGYPT

It is the purpose of this paper to add new records to the occurrence of fungi in Egypt. Those listed have been isolated for the first time from Egypt and are deposited in the writer's mycological herbarium. A number of them have also been sent to the Commonwealth Mycological Institute, Kew, England, for confirmation.

Allomyces cystogenus Emerson, from soil.

Actinomucor repens Schroet., from soil in the vicinity of cotton roots.

Mucor hiemalis Wehmer, from soil.

Monascus purpureus Wint., from soft cheese.

Sordaria fimicola (Rob.) Ces. & De Not., from seeds of beets.

Gibberella fujikuroi (Saw.) Wollenweber, from roots of onions.

Geotrichum sp., on damp paper.
Trichothecium roseum Link, on tomato fruits.
Penicillium brefeldianum Dodge, from soil.
Aspergillus luchuensis Inui, from soil.
Spicaria divaricata (Thom) Gilman & Abbott, from neroli water.
Nigrospora sp., from soil.
Melanospora zamiae Corda, on broad-bean pods.
Fusarium equiseti (Corda) Saccardo, from tomato fruits.
Epicoccum purpurascens Ehrenb., from soil.
Rhizoctonia choussii Grand & Arrillaga, from soil.—M. A. RAGAB, Department of Botany, College of Agriculture, Cairo University.

A NEW MICROBIOLOGICAL JOURNAL

The first number of the Giornale di Microbiologia (July–August, 1955) has appeared. This a multilingual journal welcoming contributions in modern languages dealing with cellular physiology, biochemistry, biophysics, genetics, and related subjects. While the first number suggests that studies on bacteria and viruses will constitute an important part of the contents, there are two papers on actinomycetes and there seems to be no reason why suitable papers on certain aspects of fungi would not be acceptable.

The subscription office is Casella Postale 3279, Milano, Italy, and the annual subscription, per six numbers, outside of Italy, is L. 3000 (about $4.75).

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MANUSCRIPT

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